Synthesis and Bioactivity of Albicidin Analogues with Amide Bond Isosteres

2021.10.02 Literature Seminar
B4 Hiromu Kakizawa

Contents

Contents

Albicidin (1)

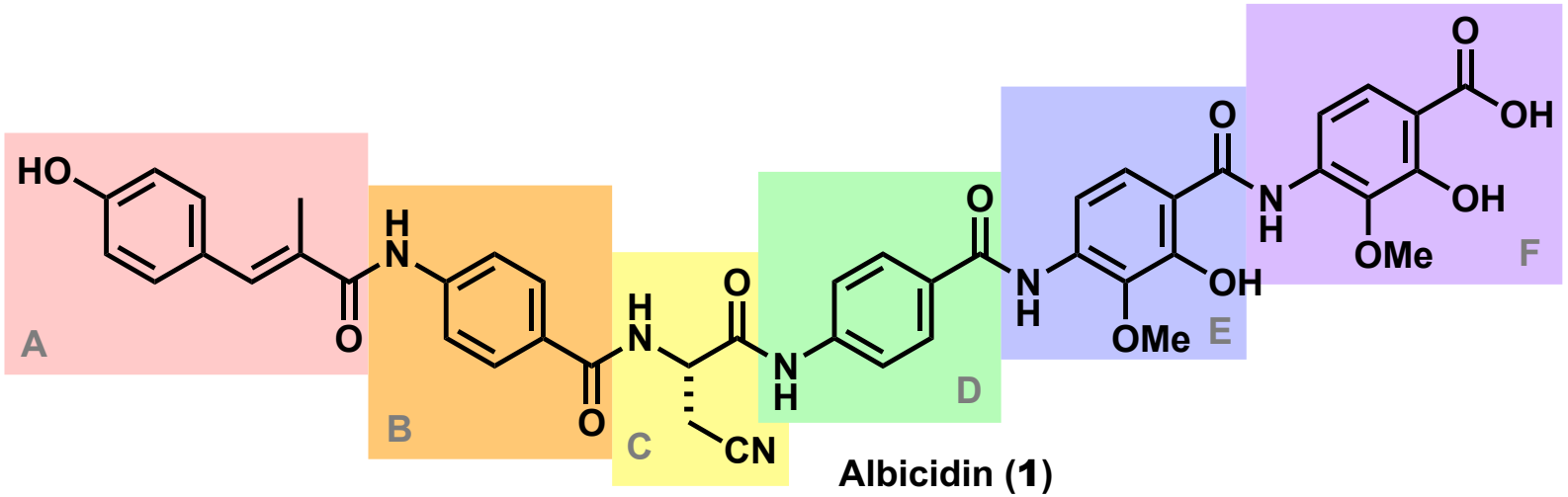
- **Isolated in 1985, from *Xanthomonas albilineans* (by Patil)** [1)]

- **Structure determination and total synthesis in 2015 (by Süssmuth)** [2) 3)]

Acyl pentapeptide composed of six building blocks (A-F), mainly aromatic characters.

- **DNA gyrase inhibition activity:** [4)]

Antibacterial activity at nanomolar concentrations against a range of gram-positive and gram-negative bacteria.
Also blocks chloroplast of plants, causing sugarcane leaf scald.



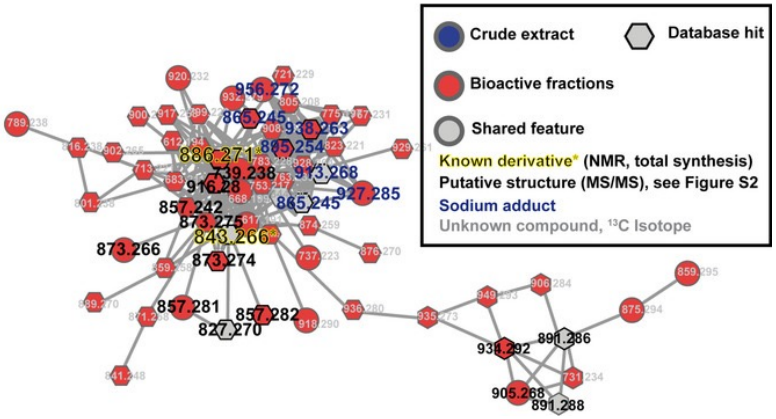
Albicidin (1)

1) Birch, R; Patil, S. *J. Gen. Microbiol.* **1985**, *131*, 1069-1075. 2) Cociancich, S.; Pesic, A.; Petras, D.; Uhlmann, S.; Kretz, J.; Schubert, V.; Vieweg, L.; Duplan, S.; Marguerettaz, M.; Noëll, J.; Pieretti, I.; Hügelland, M.; Kemper, S.; Mainz, A.; Rott, P.; Royer, M.; Süssmuth, R. *Nat. Chem. Biol.* **2015**, *11,* 195-197 3) Krets, J.; Kerwat, D.; Schubert, V.; Grätz, S.; Pesic, A.; Semsary, S.; Cociancich, S.; Royer, M.; Süssmuth, R. *Angew. Chem. Int. Ed.* **2015**, *54*, 1969-1973. 4) Hashimi, S.; Wall, M.; Smith, A.; Maxwell, A.; Birch, R. *Antimicrob. Agents Chemother.* **2007**, *51*, 181-187.

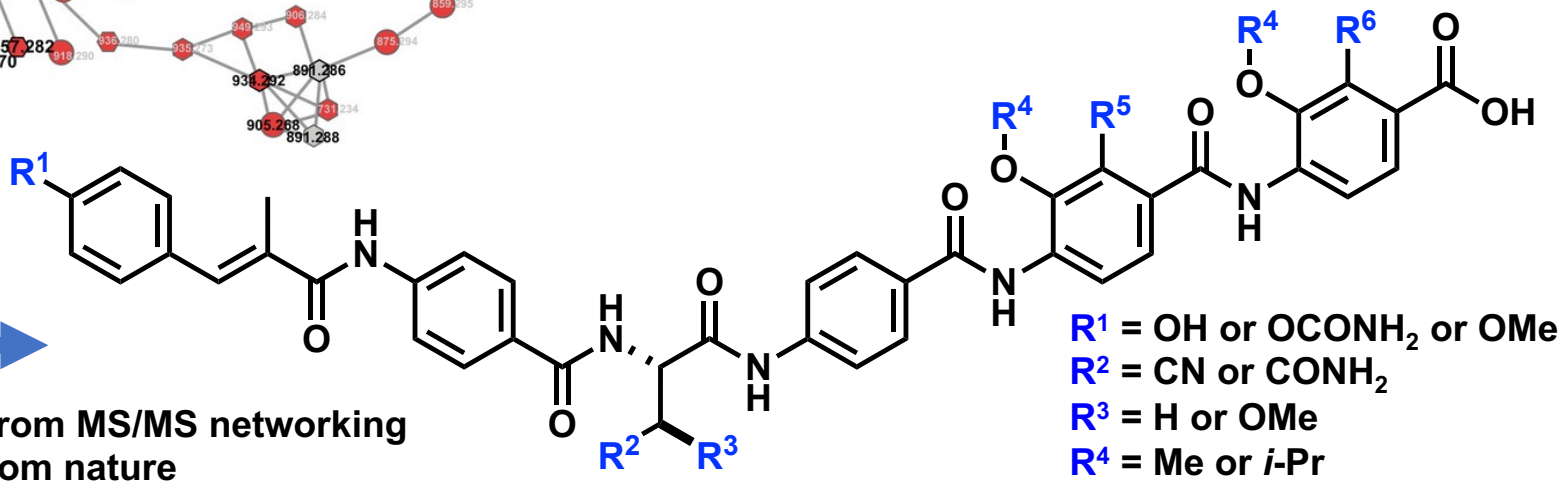
Various Analogues of **1** with Compatible antibacterial activity

At least 10 analogues of 1 have been discovered in nature so far, partly using mass spectrometric networking.[1) 2)]

Like albicidin (1), many of these analogues exhibit antibacterial activity, implying biological value of this structure.



Similar MS/MS spectra are connected through lines and form molecular networks. Near the center is the original albicidin, and its analogues are detected as nodes.



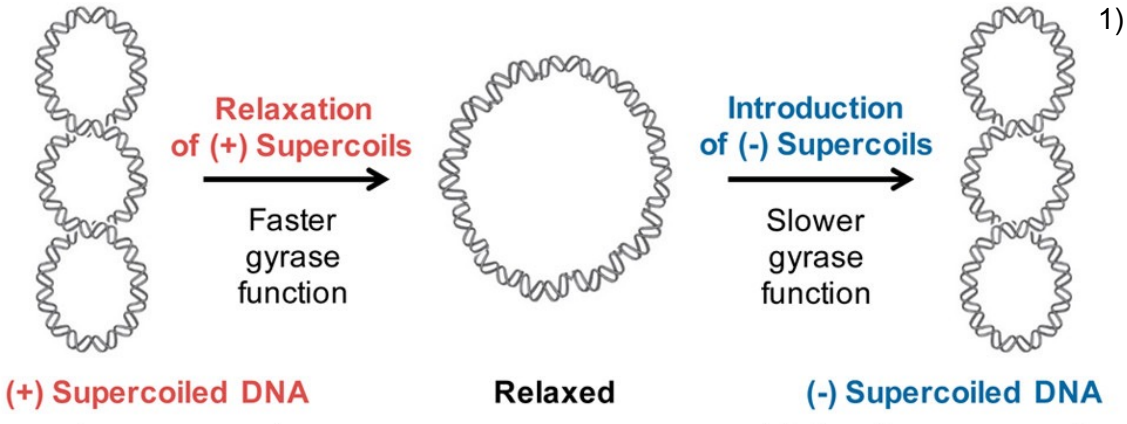
Discovered from MS/MS networking or isolated from nature

R^1 = OH or $OCONH_2$ or OMe
R^2 = CN or $CONH_2$
R^3 = H or OMe
R^4 = Me or *i*-Pr
R^5, R^6 = H or OH

1) Eckardstein, L.; Petras, D.; Dang, T.; Cociancich, S.; Sabri, S.; Grätz, S.; Kerwat, D.; Seidei, M.; Pesic, A.; Dorrestein, P.; Royer, M.; Weston, J.; Süssmuth, R. *Chem. Eur. J.* **2017**, *23*, 15316-15321. 2) Cheng, B.; Müller, R.; Trauner, D. *Angew. Chem. Int. Ed.* **2017**, *56*, 12755-12759.

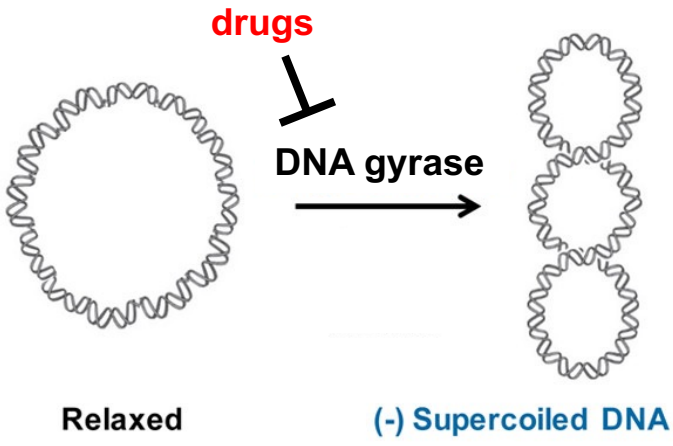
DNA Gyrase as a Drug Target

DNA gyrase is an enzyme for the relaxation of positive supercoils of DNA and the introduction of negative supercoils to DNA.



1)

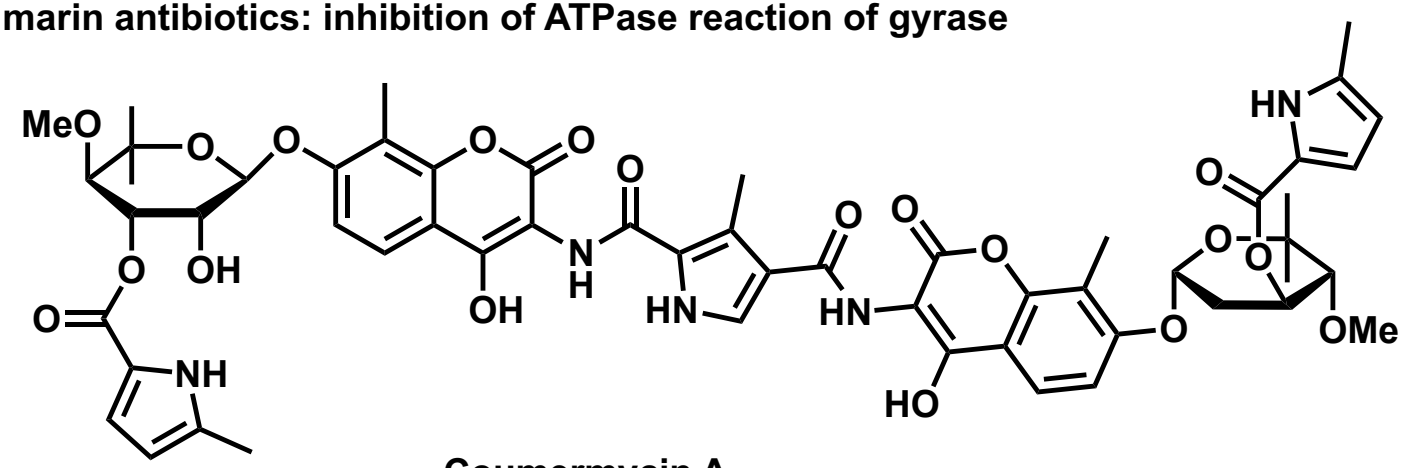
Negative supercoils of DNA are essential in DNA replication and transcription, and therefore the inhibition of DNA gyrase leads to cell death. There are some molecules which selectively inhibit DNA gyrase of bacteria and do not affect mammalian cells, and thus DNA gyrase has been regarded as a drug target.



1) Ashley, R.; Blower, T.; Berger, J.; Osheroff, N. Biochemistry. **2017**, *56*, 5440-5448.

Inhibition of DNA Gyrase

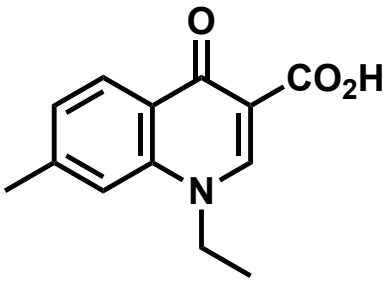
Coumarin antibiotics: inhibition of ATPase reaction of gyrase



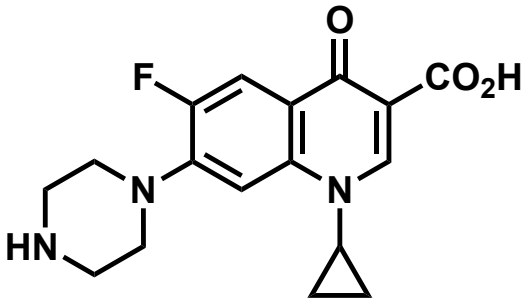
Coumermycin A_1

Resistance mutation: *gyrB*

Quinolone antibiotics: formation of gyrase-quinolone-DNA complex



Nalidixic acid



Ciprofloxacin

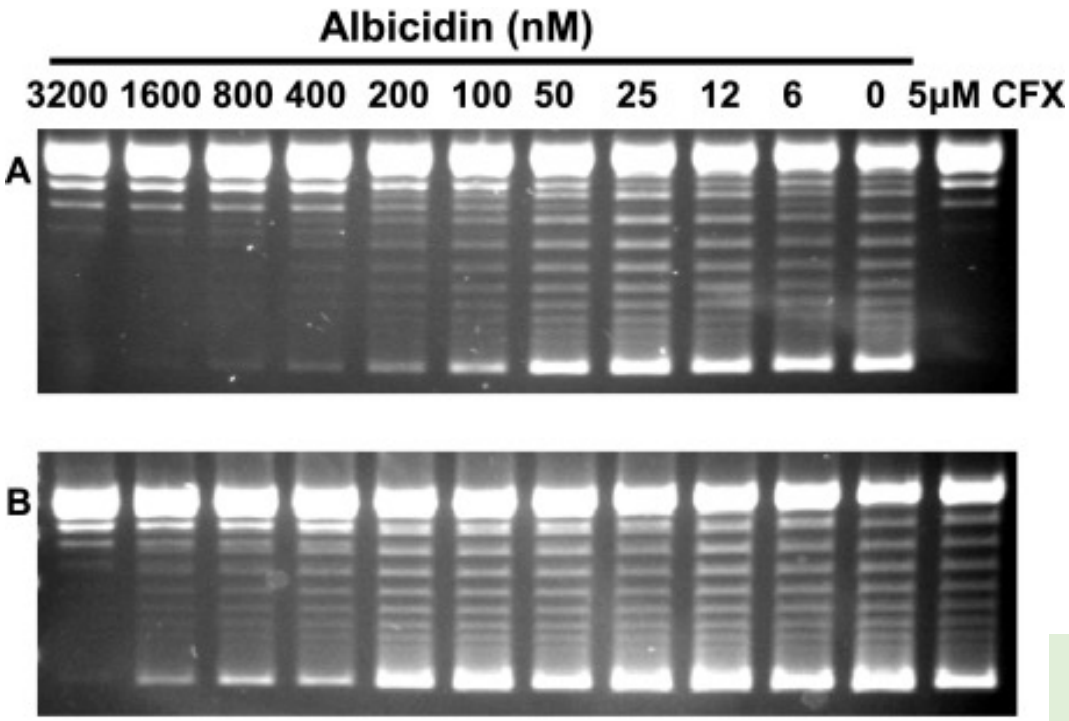
Resistance mutation: *gyrA* and *gyrB*

These exhibit antibacterial activity against a range of bacteria, but due to development of antibacterial resistance, there has been an urgent need for novel antibiotics.

1) Collin, F.; Karkare, S.; Maxwell, A. *Appl. Microbiol. Biotechnol.* **2011**, *92*, 479–497.

DNA Gyrase Inhibition Activity of **1**

Inhibition assay of DNA gyrase



Each concentrations of Albicidin (1) + relaxed DNA +
A: DNA gyrase (*E. coli* MG1665 wild type)
B: DNA gyrase (quinolone-resistant *E. coli* mutant)

CFX lane: ciprofloxacin instead of 1

< upper band: Relaxed DNA

< lower band: Supercoiled DNA

Disappearance of lower band represents successful inhibition of DNA gyrase.

1 inhibited supercoiling of DNA catalyzed by *E. coli* DNA gyrase, with an IC_{50} ≈ 40 nM.
Although the efficiency declined (IC_{50} ≈ 200 nM), 1 also inhibited DNAgyrase from quinolone-resistant *E. coli*.

1) Hashimi, S.; Wall, M.; Smith, A.; Maxwell, A.; Birch, R. Antimicrob. Agents Chemother. **2007**, *51*, 181-187.

Antibacterial Activity of **1**

Antibacterial activity (with ciprofloxacin, a fluoroquinolone antibiotic)

Strain	MIC value (μg/mL)	
	1	Ciprofloxacin
E. coli	0.031	0.015
E. coli (*qnrA1* mutation*)	0.063	32
E. coli (*gyrA1* mutation*)	0.5	> 64
S. enteritidis	0.5	0.031
P. aeruginosa	1	0.5
S. aureus	16	0.5
M. luteus	1	not determined

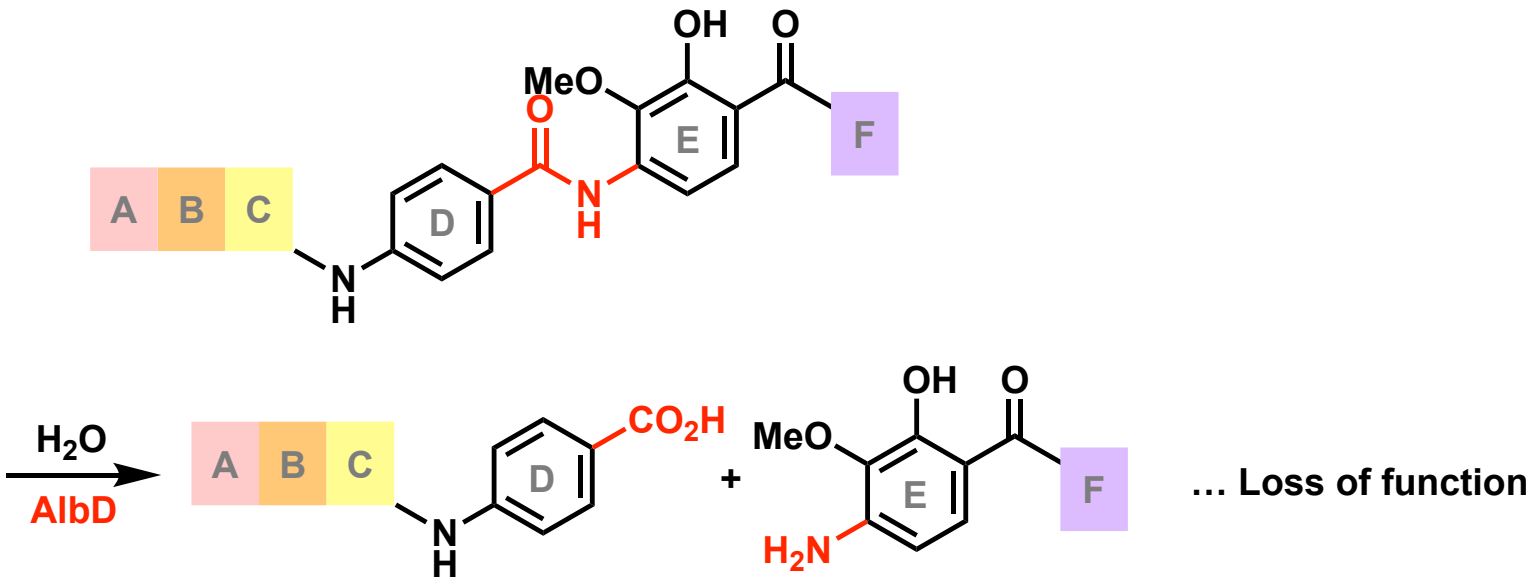
* fluoroquinolone resistant mutations

Albicidin (1) exhibited potent antibacterial activity against the fluoroquinolone-resistant bacteria strains as well.

1) Krets, J.; Kerwat, D.; Schubert, V.; Grätz, S.; Pesic, A.; Semsary, S.; Cociancich, S.; Royer, M.; Süssmuth, R. *Angew. Chem. Int. Ed.* **2015**, *54*, 1969-1973.

Enzymatic Hydrolysis of 1 by AlbD

AlbD (Albicidin detoxification enzyme): endopeptidase expressed in *Pantoea dispersa*



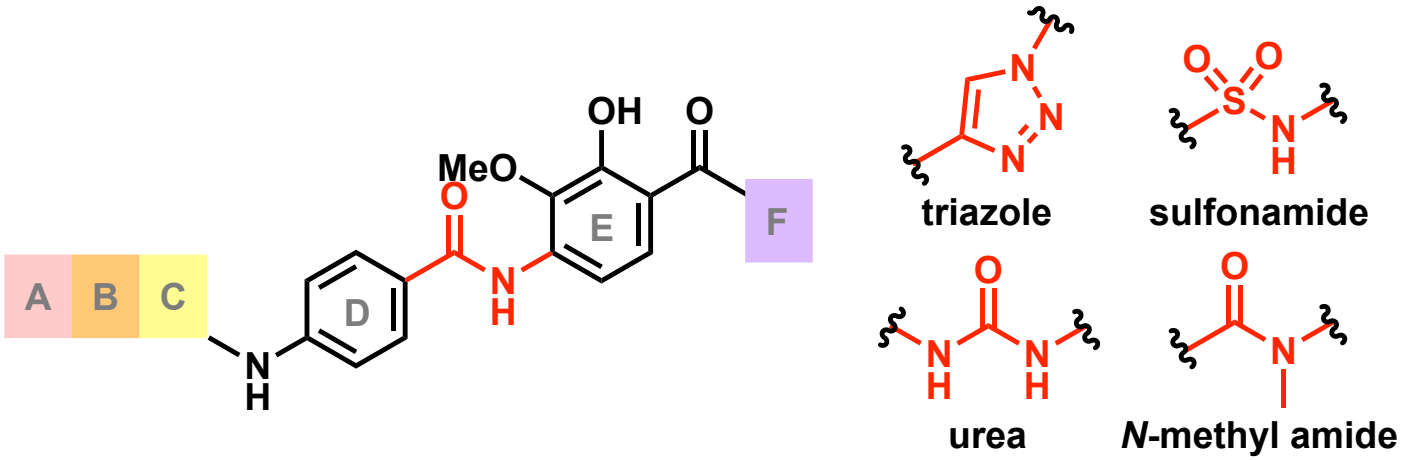
… Loss of function

AlbD catalyzes hydrolysis of amide bond between D and E building blocks, causing a complete loss of DNA gyrase inhibition activity of albicidin.

1) Vieweg, L.; Krets, J.; Pesic, A.; Kerwat, D.; Grätz, S.; Royer, M.; Cociancich, S.; Mainz, A.; Süssmuth, R. *J. Am. Chem. Soc.* **2015**, *137*, 7608-7611.

Attempted Substitution of D-E Linkage

To overcome AlbD, four analogues with substituted D-E linkage were synthesized.



MIC values (μg/mL) of 1 and 1 analogues

	original (1)	triazole	sulfonamide	urea	*N*-methyl amide
E. coli DSM1116	0.063	0.063	8.0	2.0	8.0
S. typhimurium TA100	0.063	0.125	0.50	8.0	8.0
S. subtillis DSM10	0.25	4.0	8.0	8.0	8.0

*** The bacteria species used in this assay do not express AlbD.**

Although substituting the AlbD-labile amide bond avoided hydrolysis by AlbD, it led to a decreased antibacterial activity. Further exploration for isosteres remained necessary.

1) Behroz, I.; Durkin, P.; Grätz, S.; Seidel, M.; Rostock, L.; Spinczyk, M.; Weston, J.; Süssmuth, R. *Chem. Eur. J.* **2019**, *25*, 16538-16543.

Contents

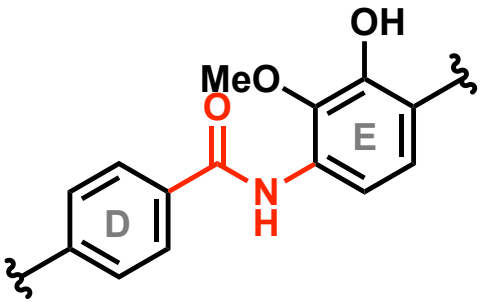
Prof. Roderich Süssmuth

1995	B.S. @ University of Tübingen (Professor G. Jung)
1996-	Research Associate @ Institute of Organic Chemistry, University of Tübingen
1999	Ph.D. @ University of Tübingen (Professor G. Jung)
2000-	Visiting Scientist Fellowship @ The Scripps Research Institute (Professor R.A. Lerner and Prof. C.F. Barbas)
2001-	Emmy-Noether-Research Fellow @ University of Tübingen
2004-	Associate Professor @ Technical University Berlin
2008-	Full Professor @ Technical University Berlin

Research interests:
- Bioactive secondary metabolites
- Total synthesis of natural compounds
- Structure-activity relationships for a design of potent future drugs

Further Exploration for Isosteres

Isosteric replacements of D and E building blocks

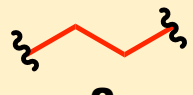


origial albicidin (**1**)

- Alkane linkage (**2**)
- Alkene linkage (**3**)
- Alkyne linkage (**4**)

… For enhanced stability against chemical and enzymatic hydrolysis

Isosteres



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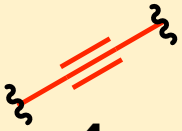

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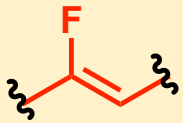

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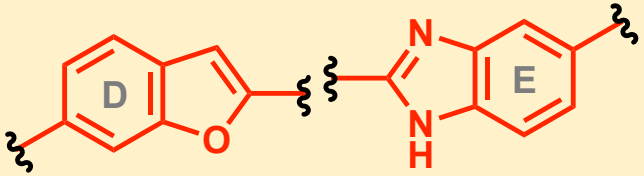


6 **7**

- Fluoroalkene linkage (**5**)

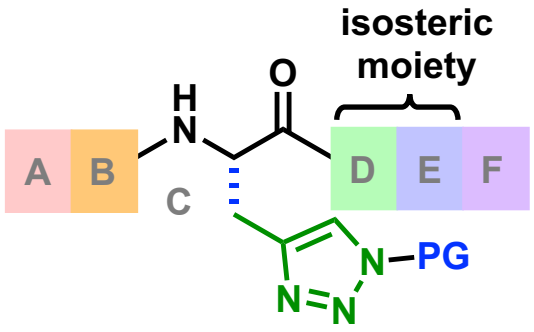
… A well-known isostere of amide bond with sterically and electrically similarity

- D ring substituted with benzofuran (**6**)
- E ring substituted with benzimidazole (**7**)

… For reduced structural flexibility

Synthesis of Six Isosteres

Albicidin isosteres (2-7) to be synthesized



aza-his albicidin (1')

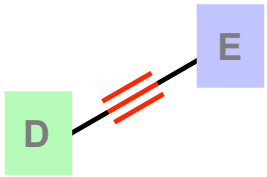
PG = Protecting Group

***Aza-histidine** was used as C building block instead of Cyanoalanine, which have an optimized antibacterial profile and a higher chemical stability.** [1)]

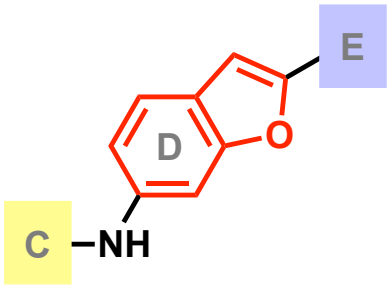
Synthetic method for each isosteric moiety

X = H or F

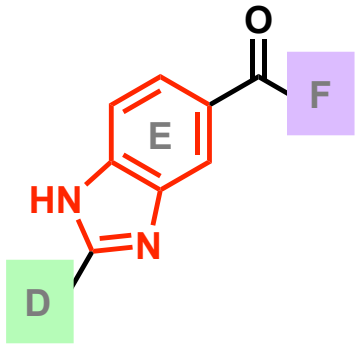
Alkane (2), Alkene (3), Fluoroalkene (5): Pd-catalyzed cross coupling



Alkyne (4): Pd-Cu-catalyzed Sonogashira coupling



Benzofuran (6): Sonogashira coupling followed by Pd-Cu-catalyzed intramolecular annulation

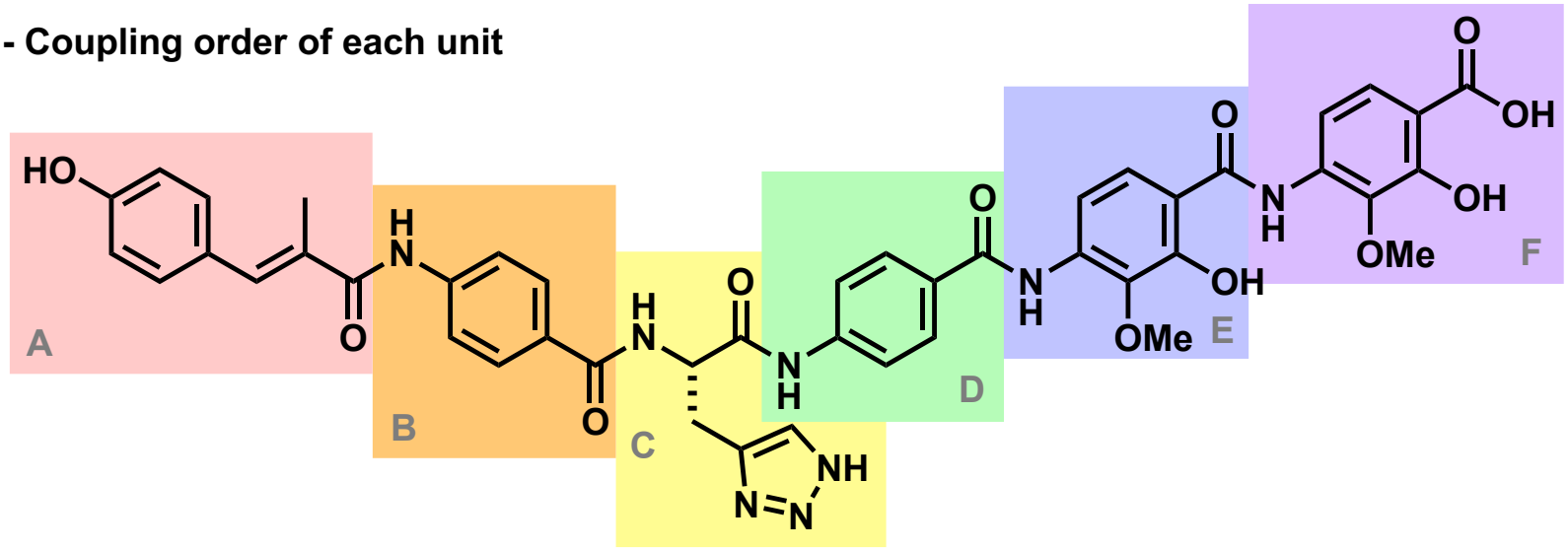


Benzimidazole (7): Reaction between *o*-phenylenediamine and benzaldehyde

1) Behroz, I.; Durkin, P.; Grätz, S.; Seidel, M.; Rostock, L.; Spinczyk, M.; Weston, J.; Süssmuth, R. *Chem. Eur. J.* **2019**, *25*, 16538-16543.

Strategies for the Synthesis of 1 Analogues (1)

- Coupling order of each unit

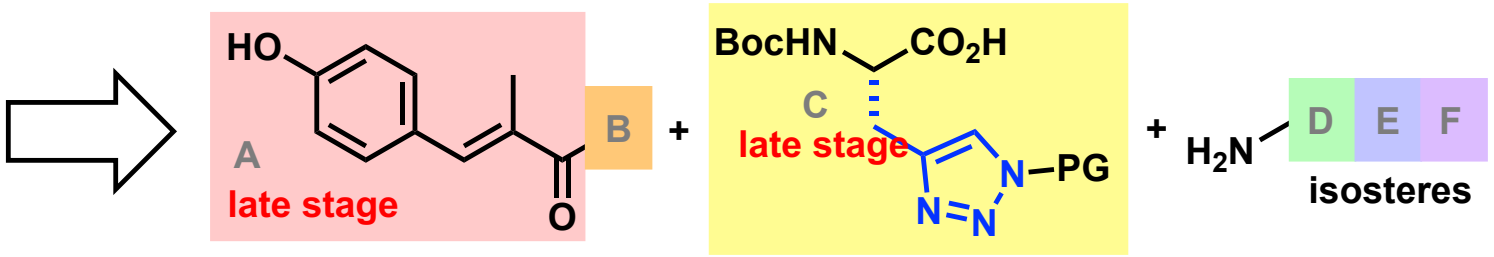


restricted solubility by the introduction of A block [1)]

risk of racemization of C block during the reactions [2)]

Introduction of these moiety in the late stage enabled to minimize the risk of troubles.

analogues 2-7



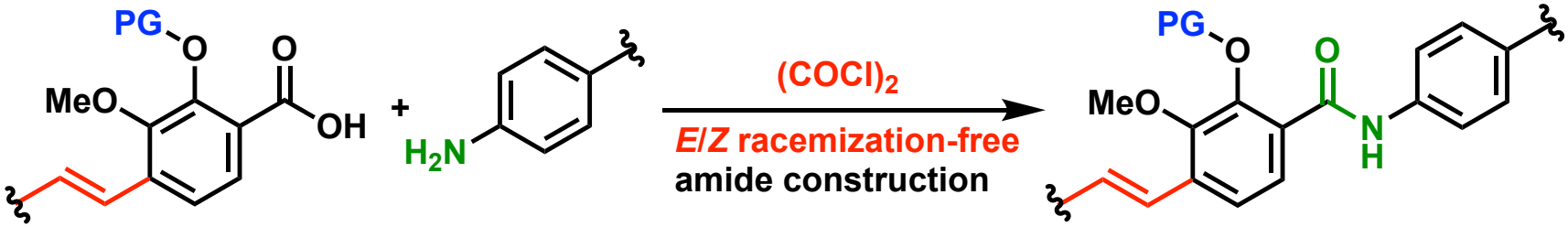
1) Krets, J.; Kerwat, D.; Schubert, V.; Grätz, S.; Pesic, A.; Semsary, S.; Cociancich, S.; Royer, M.; Süssmuth, R. *Angew. Chem. Int. Ed.* **2015**, *54*, 1969-1973. 2) Eckardstein, L.; Petras, D.; Dang, T.; Cociancich, S.; Sabri, S.; Grätz, S.; Kerwat, D.; Seidei, M.; Pesic, A.; Dorrestein, P.; Royer, M.; Weston, J.; Süssmuth, R. *Chem. Eur. J.* **2017**, *23*, 15316-15321.

Synthetic Strategies for the of 1 Analogues (2)

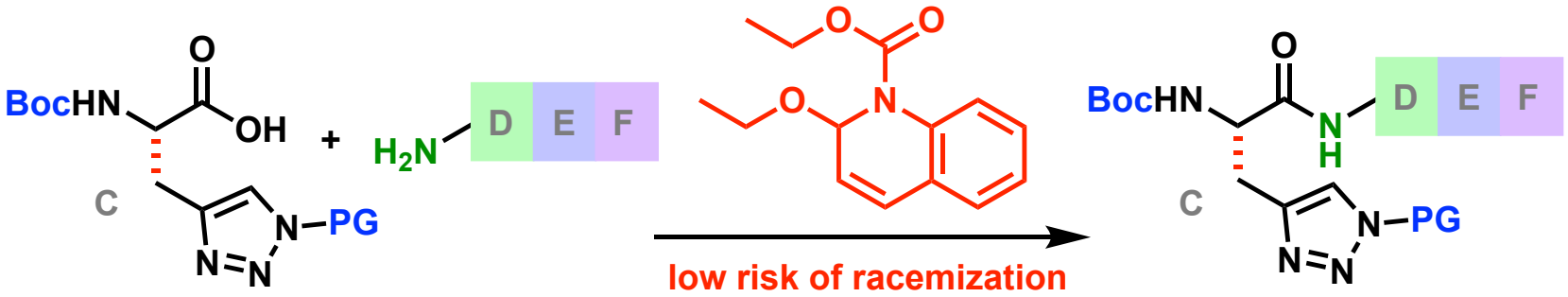
- Strategy of condensation reagent

Low nucleophilicity of aromatic amine required harsh conditions for amide formation.
At the same time, it was necessary to avoid *E*/*Z* isomerization and racemization in some substrates.

To avoid *E*/*Z* isomerization…
Relativey mild conditions, Oxalyl chloride $(COCl)_2$ was applied.



To avoid racemization…
EEDQ was applied, which is known for its low rates of racemization.[1)]



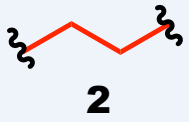
1) Krets, J.; Kerwat, D.; Schubert, V.; Grätz, S.; Pesic, A.; Semsary, S.; Cociancich, S.; Royer, M.; Süssmuth, R. *Angew. Chem. Int. Ed.* **2015**, *54*, 1969-1973.

Synthetic Strategies for the of 1 Analogues (3)

- Protecting group strategy

For the free phenol and carboxylic acid (O-PG)

PG = Bn



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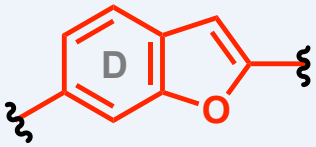
For global deprotection and hydrogenation at the same time



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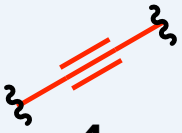
Stable under Pd-catalyzed coupling conditions

PG = Allyl

3



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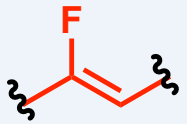

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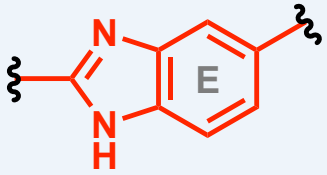

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Unstable to Bn deprotection conditions (H_2, Pd/C) due to unsaturated bonds

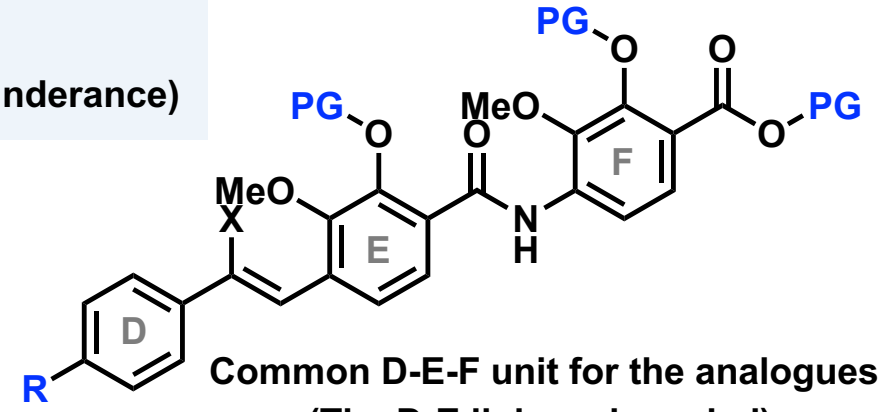
PG = MOM, *t*-Bu, etc. (acid-labile protecting groups)

Not applied (very inefficient deprotection for steric hinderance)

For the amine moiety (R)

NO_2 group was applied

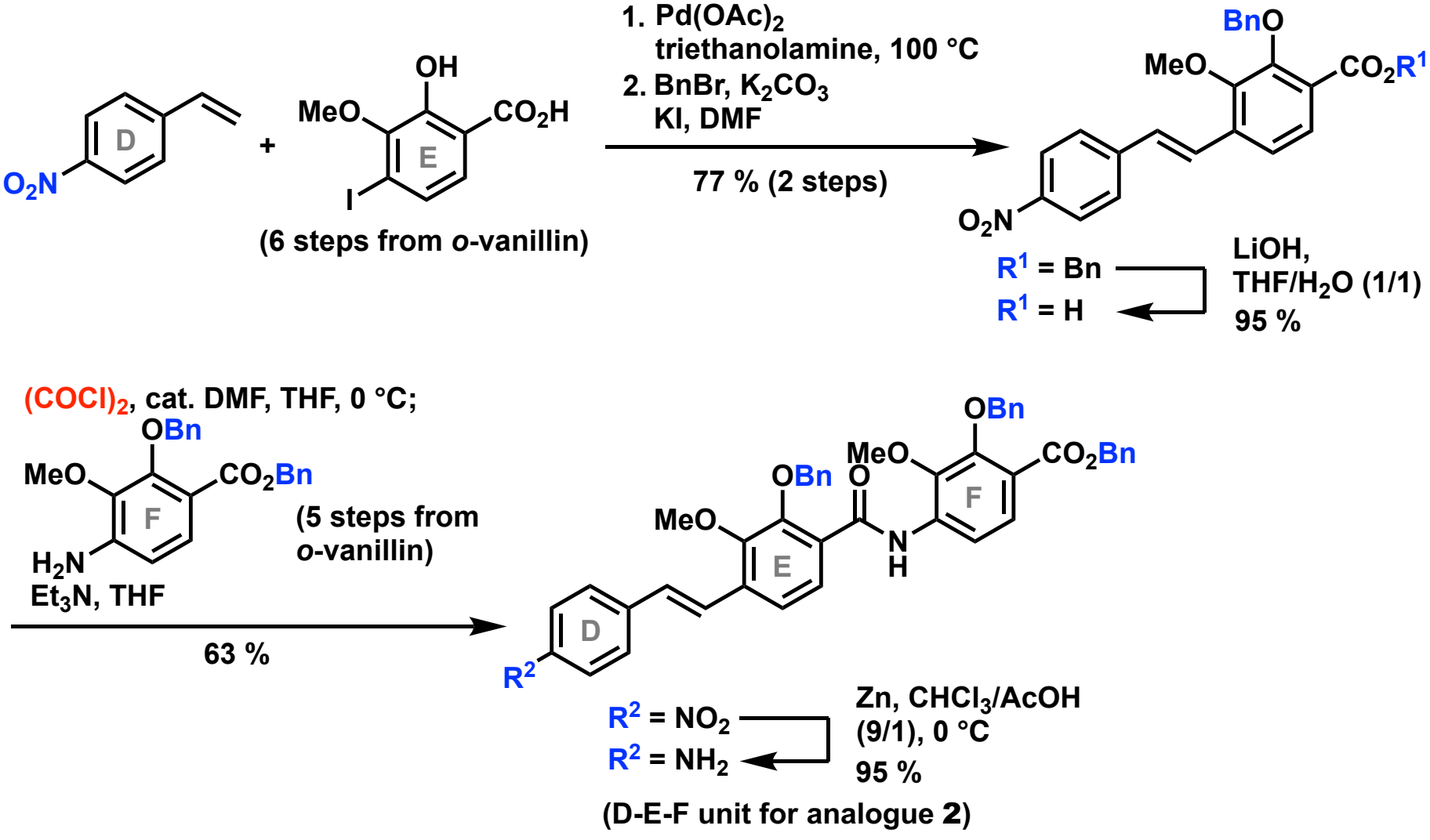
(For atom economy and deprotection under mild conditions (Zn, AcOH))



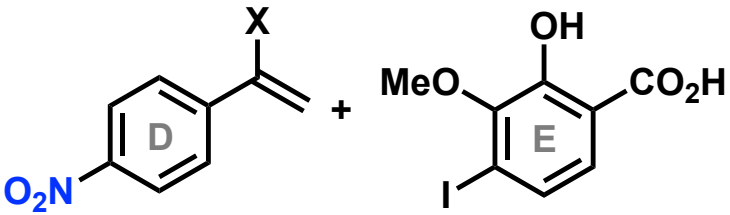
Common D-E-F unit for the analogues

(The D-E linkage is varied)

Construction of D-E-F Unit: Alkane Isostere (2)



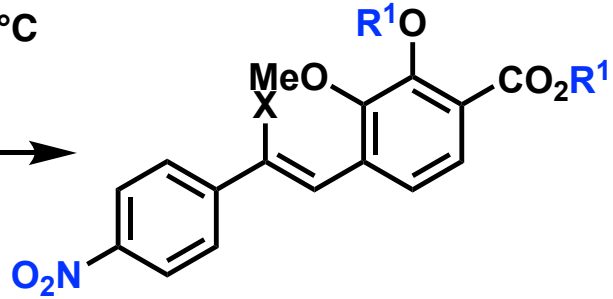
Construction of D-E-F Unit: Alkene And Fluoroalkene Isosteres (3 and 5)



X = H (for **3**)
X = F (for **5**)

1. $Pd(OAc)_2$
   triethanolamine, 100 °C
2. allyl bromide
   K_2CO_3, KI, DMF

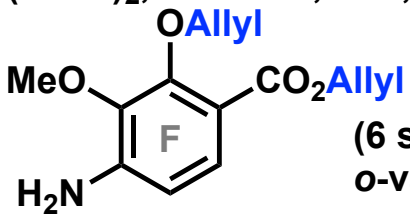
3: 50 %, **5**: 60 %
(2 steps)



R^1 = Allyl
R^1 = H

LiOH,
THF/H_2O (1/1)
3: 96 %, **5**: 96 %

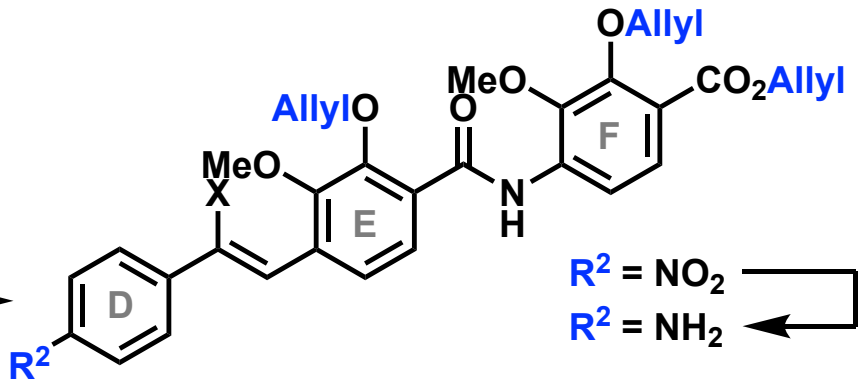
$(COCl)_2$, cat. DMF, THF, 0 °C;



Et_3N, THF

(6 steps from *o*-vanillin)

3: 75 %, **5**: 44 %

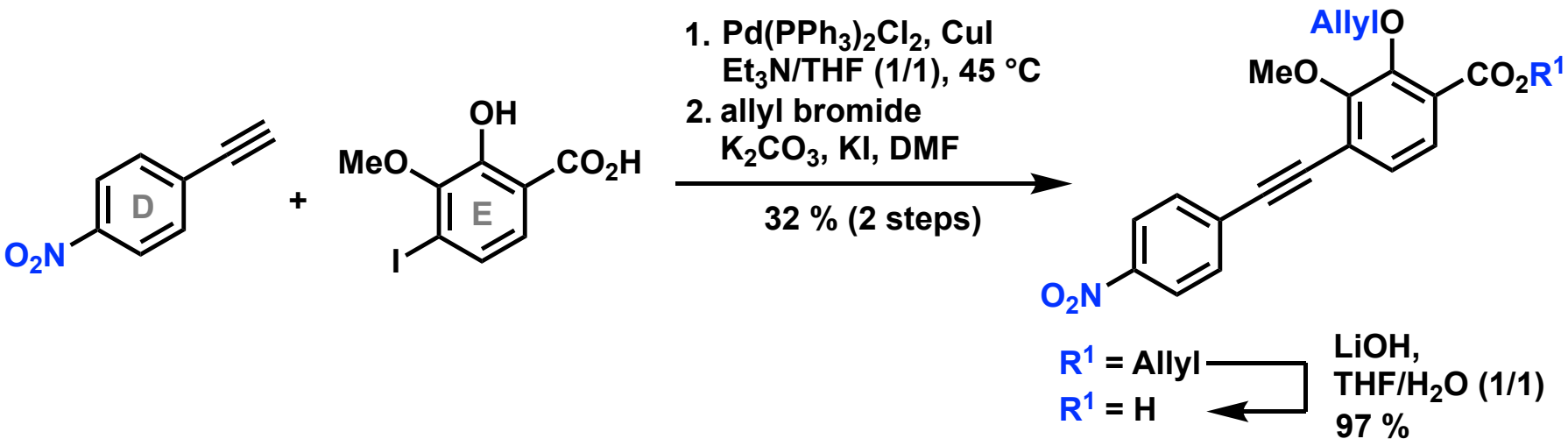


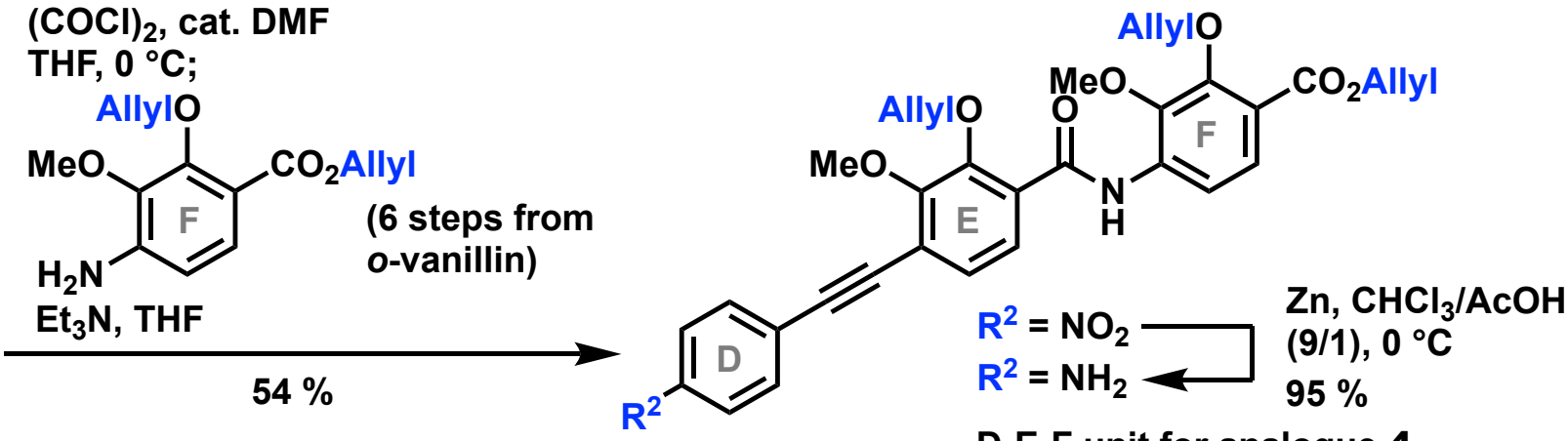
R^2 = NO_2
R^2 = NH_2

Zn, $CHCl_3$/AcOH
(9/1), 0 °C
3: 95 %, **5**: 92 %

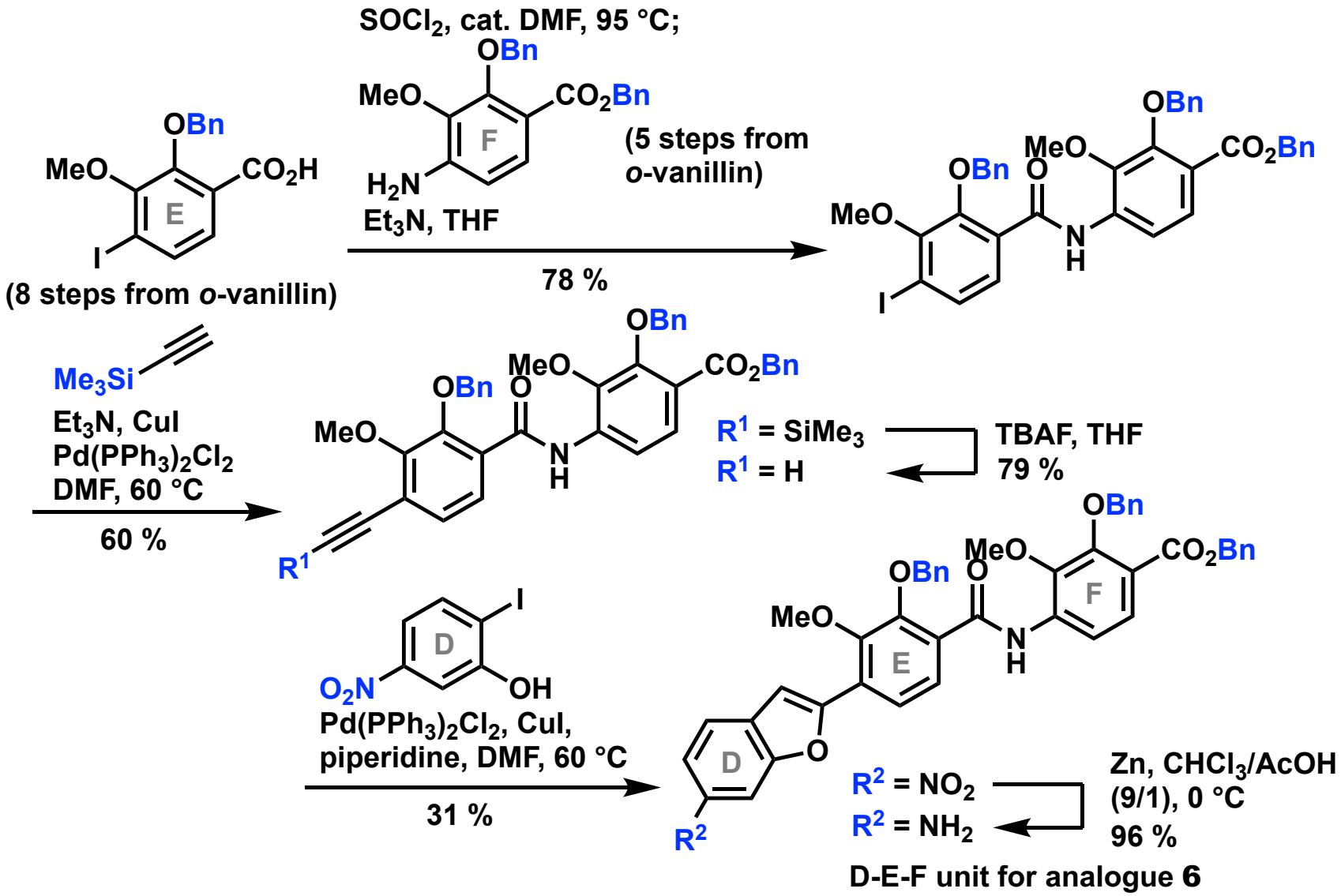
X = H: D-E-F unit for analogue **3**
X = F: D-E-F unit for analogue **5**

Construction of D-E-F Unit: Alkyne Isostere (4)

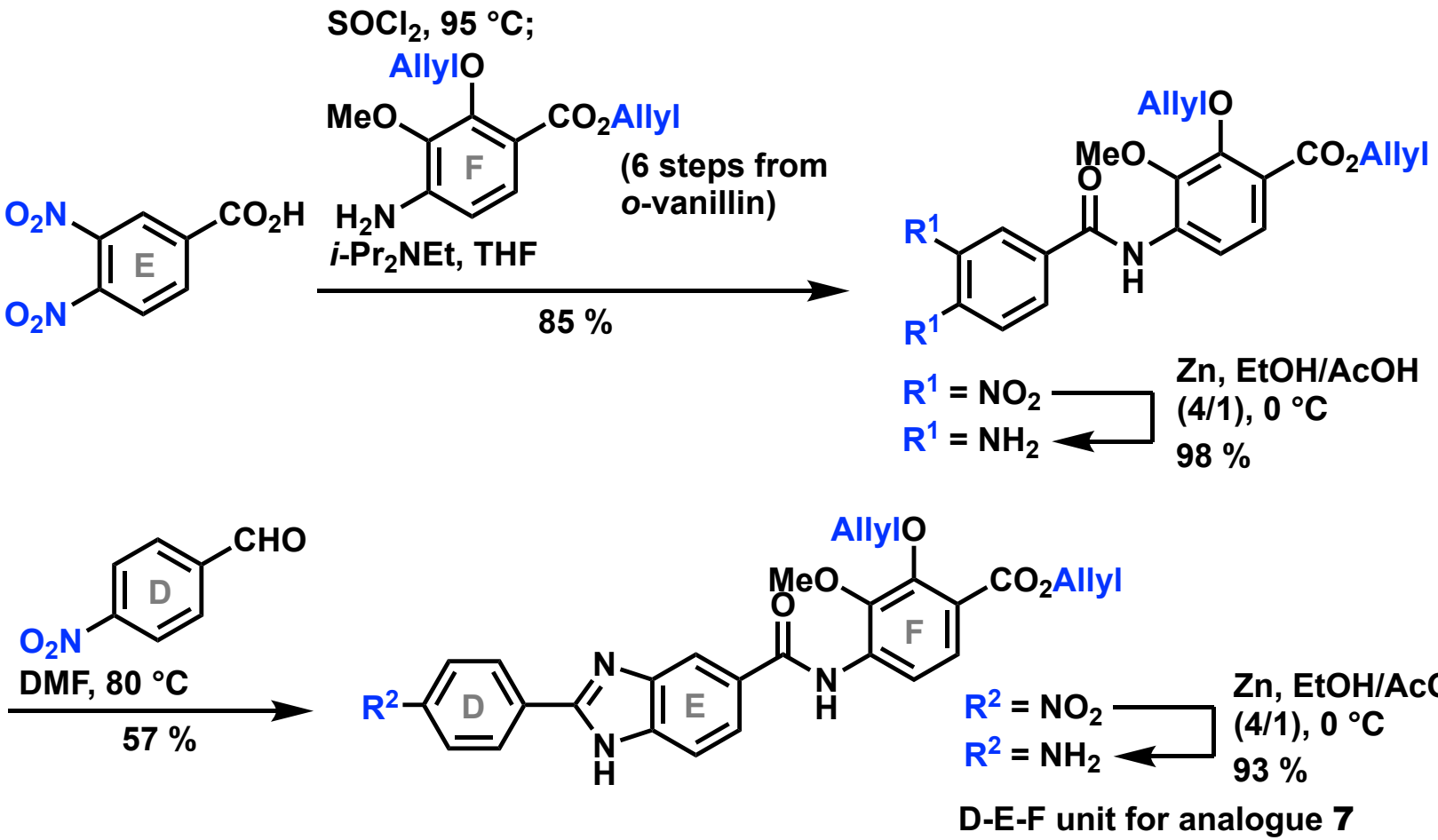




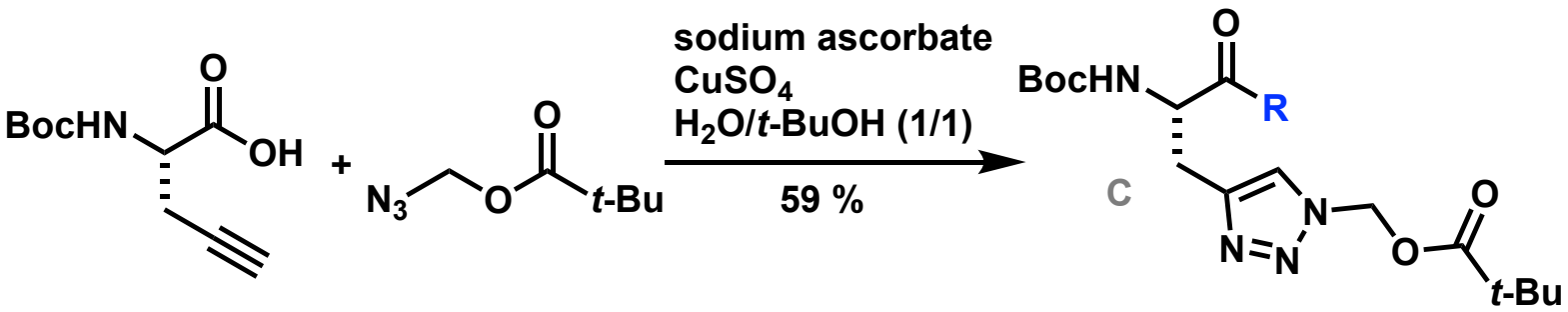
Construction of D-E-F Unit: Benzofuran Isostere (6)

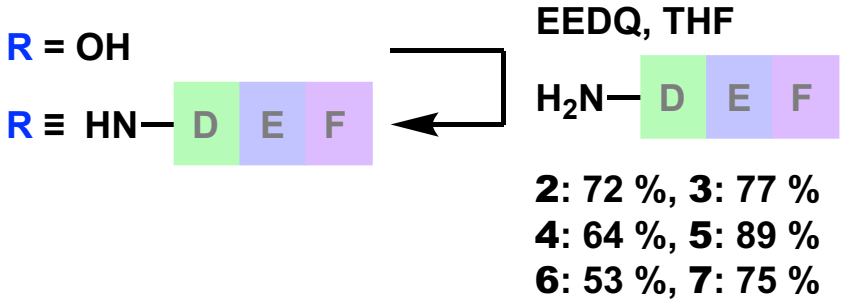


Construction of D-E-F Unit: Benzimidazole Isostere (7)



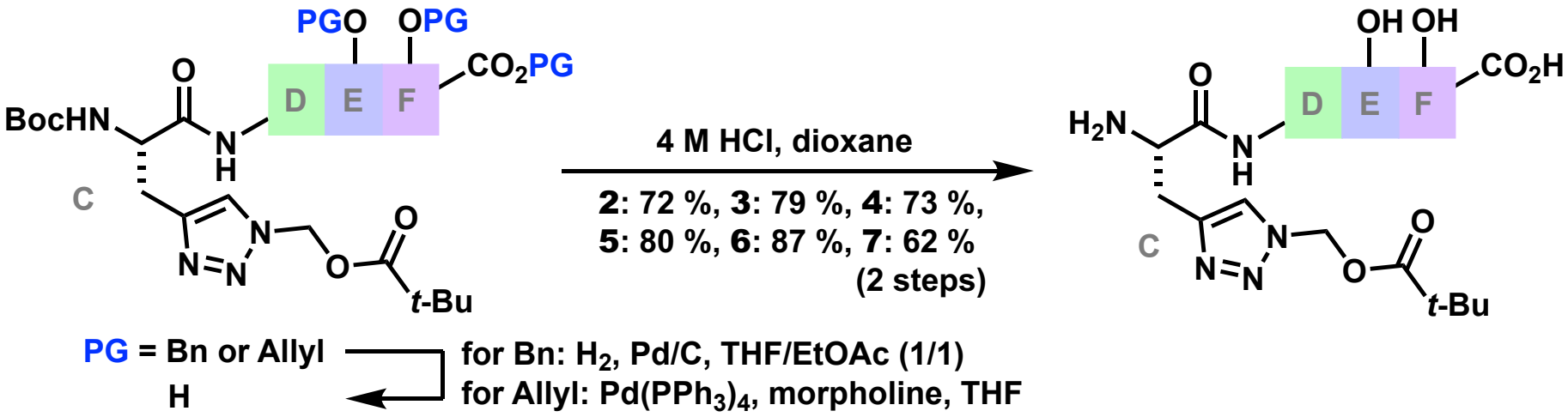
Coupling of D-E-F Unit to C Unit



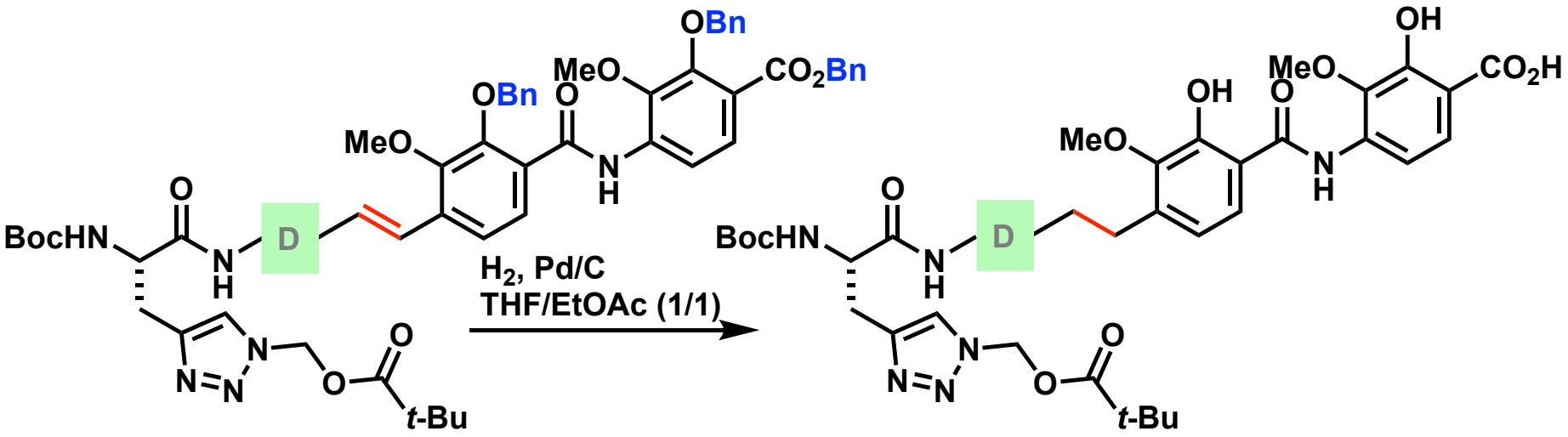


C unit was constructed via Click reaction, which was condensed with all of the six D-E-F unit of each analogues in good to moderate yield.

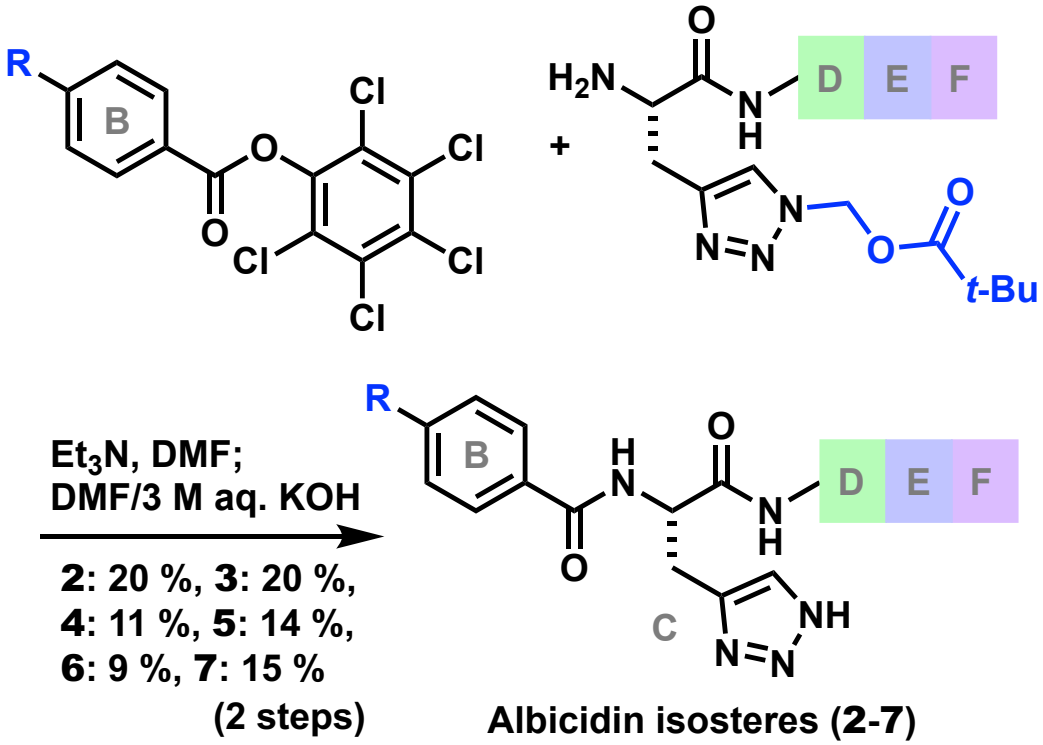
Deprotection of Allyl, Bn, And Boc Group

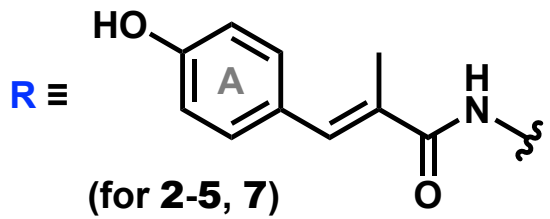


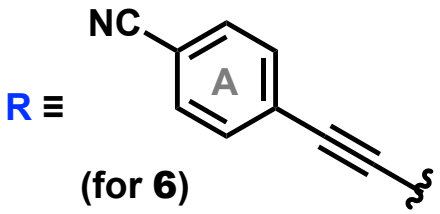
Global deprotection and hydrogenation (for isostere 2)



Coupling of A-B Unit



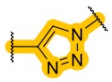
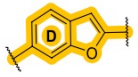
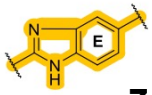




When coupling of original A-B unit was tried for benzofuran isostere, no desired compound was obtained. Therefore, an alternative A-B unit (shown above) was used.

Bioactivity of Albicidin Isosteres

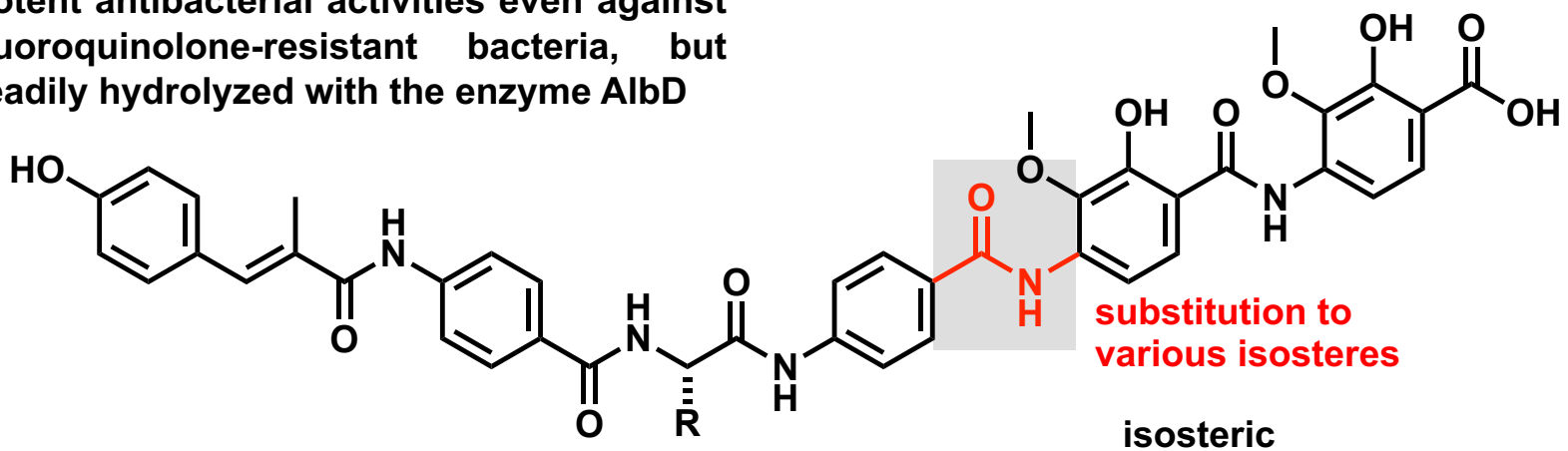
MIC value (μg/mL), stability against AlbD, and gyrase inhibition activity

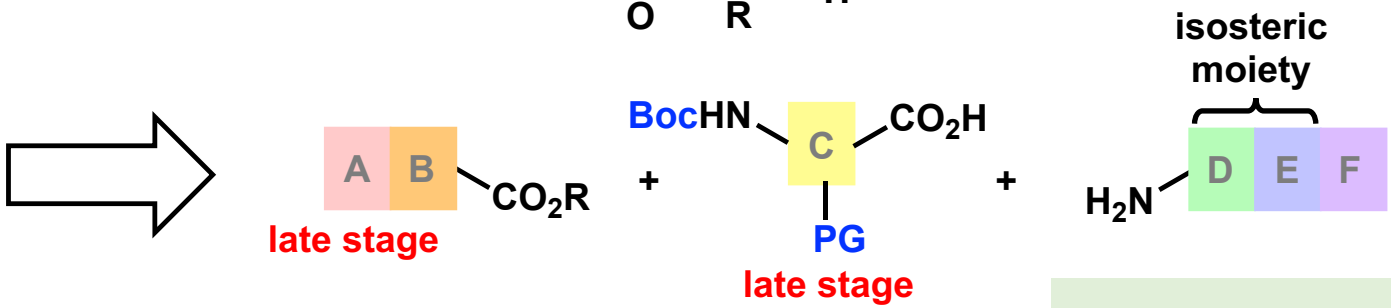
bacterial strain	 1’	 triazole	 sulfonamide	 2	 3	 4	 5	 6	 7
E. coli BW25113	0.063	0.125	8.0	0.25	0.125	0.063	0.063	1.0	0.25
S. typhimurium TA100	0.063	0.125	0.5	0.25	0.063	≤0.016	0.031	0.125	0.063
B. subtilis DSM10	0.250	4.0	8.0	0.063	0.063	0.063	0.031	0.25	2.0
M. phlei DSM750	2.0	8.0	8.0	1.0	0.50	0.50	0.250	1.0	8.0
E. coli DSM1116	0.016	0.063	8.0	0.25	0.125	0.063	0.063	2.0	0.25
M. luteus DSM1790	0.5	8.0	8.0	0.125	0.25	0.25	0.063	0.25	8.0
AlbD stability	✗	✓	✓	✓	✓	✓	✓	n.d.	✓
E. coli gyrase inhibition	n.d.	↓	↓	≈	↑	↑	↑	↓	↓

- **The isosteres showed antibacterial activity even in the presence of AlbD.**
- **Introduction of alkyne and fluoroalkene linkage increased antibacterial activity.**

Summary

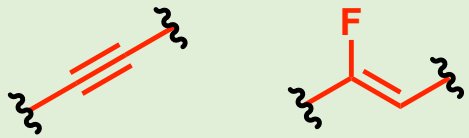
Original albicidin:
potent antibacterial activities even against fluoroquinolone-resistant bacteria, but readily hydrolyzed with the enzyme AlbD





Three synthetic strategies:
1) Coupling order of each unit
2) Careful choice of condensation reagent
3) Protecting group strategy

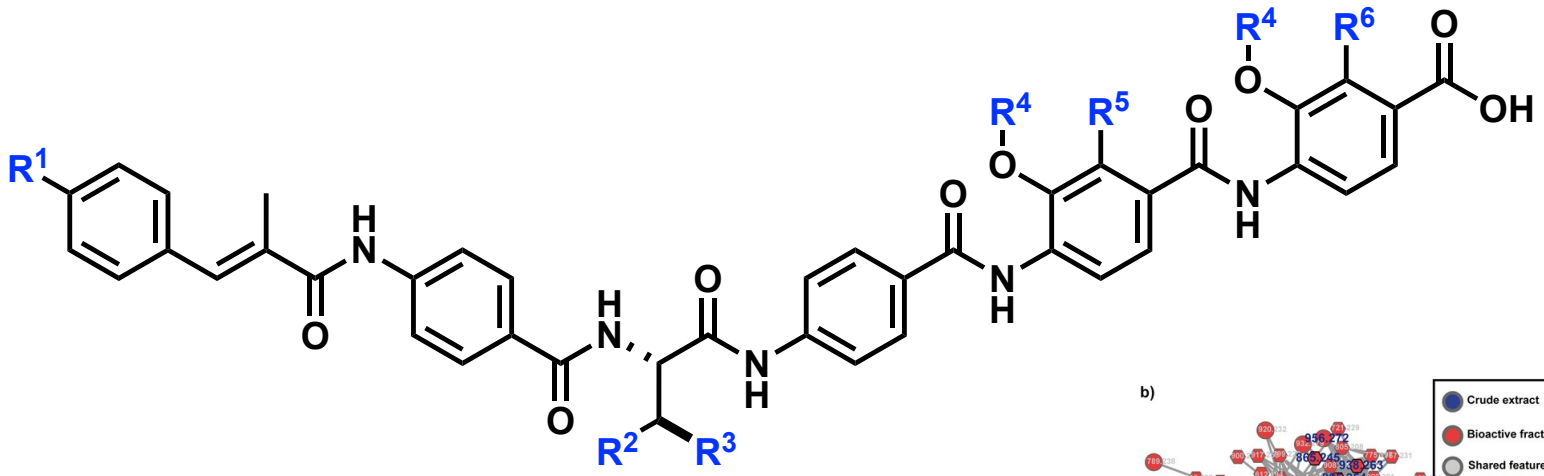
Amide isosteres which can overcome AlbD while maintaining potent antibacterial activity:

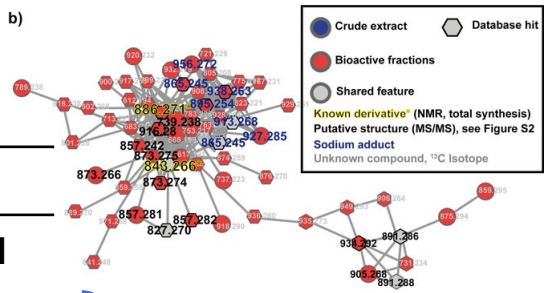


alkyne fluoroalkene

Appendix

Structures of Various Analogues of 1





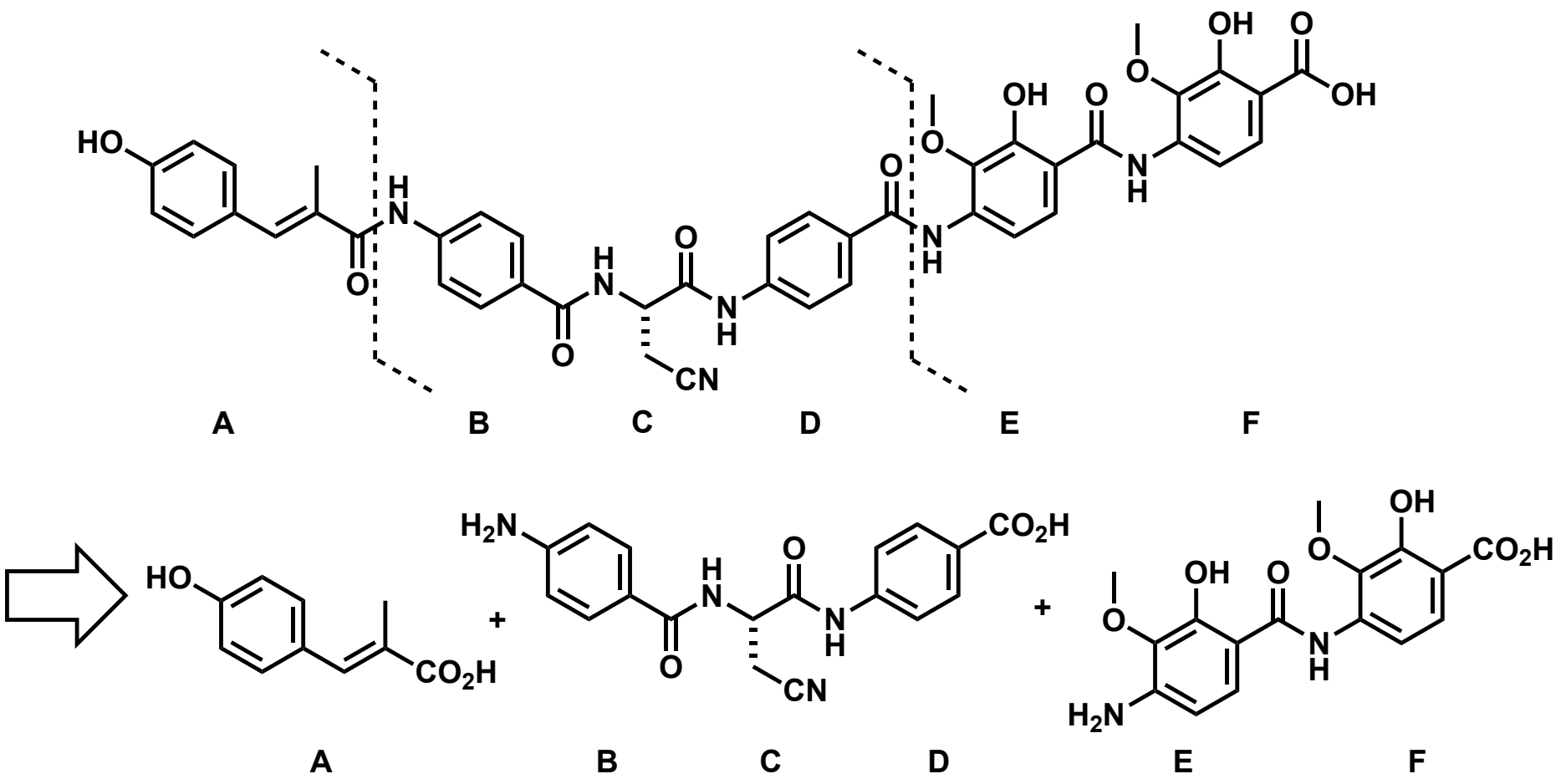
	R^1	R^2	R^3	R^4	R^5	R^6
1	OH	CN	H	Me	OH	OH
carbamoyl-1	$OCONH_2$	CN	H	Me	OH	OH
F-dehydroxy-1	OH	CN	H	Me	OH	H
β-OMe-Cya-1	OH	CN	OMe	Me	OH	OH
β-OMe-Asn-1	OH	$CONH_2$	OMe	Me	OH	OH
cystobactamid 919-2	OH	$CONH_2$	OMe	*i*-Pr	OH	H
coralmycin	OH	$CONH_2$	OMe	*i*-Pr	OH	OH

Found by mass spectrometric networking [1)]

Isolated by Kim In 2016 [2)]

1) Eckardstein, L.; Petras, D.; Dang, T.; Cociancich, S.; Sabri, S.; Grätz, S.; Kerwat, D.; Seidei, M.; Pesic, A.; Dorrestein, P.; Royer, M.; Weston, J.; Süssmuth, R. *Chem. Eur. J.* **2017**, *23*, 15316-15321. 2) Kim, Y.; Kim, H.; Kim, G.; Cho, S.; Takahashi, S.; Koshimo, H. *Nat. Prod.* **2016**, *79*, 2223-2228.

Strategy for Total Synthesis of Albicidin

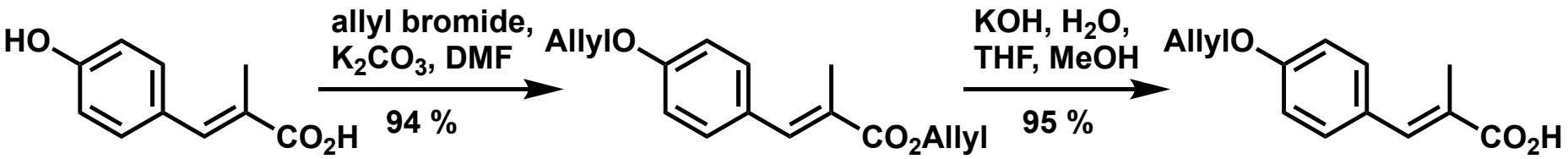


Introduction of A unit in the early stage of synthesis resulted in low solubility of compound, so A-B bond was formed in the last step.

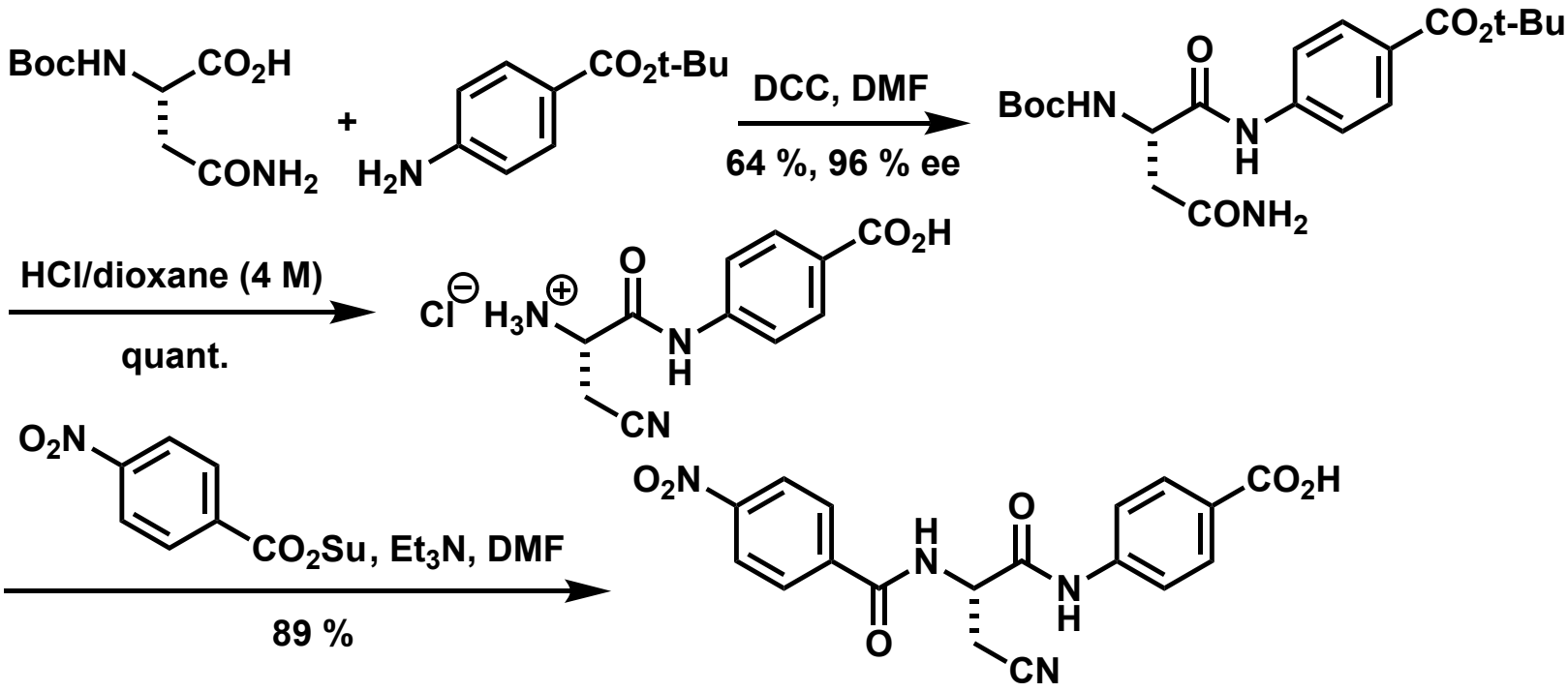
1) Krets, J.; Kerwat, D.; Schubert, V.; Grätz, S.; Pesic, A.; Semsary, S.; Cociancich, S.; Royer, M.; Süssmuth, R. *Angew. Chem. Int. Ed.* **2015**, *54*, 1969-1973.

Construction of A Unit And B-C-D Unit

A unit

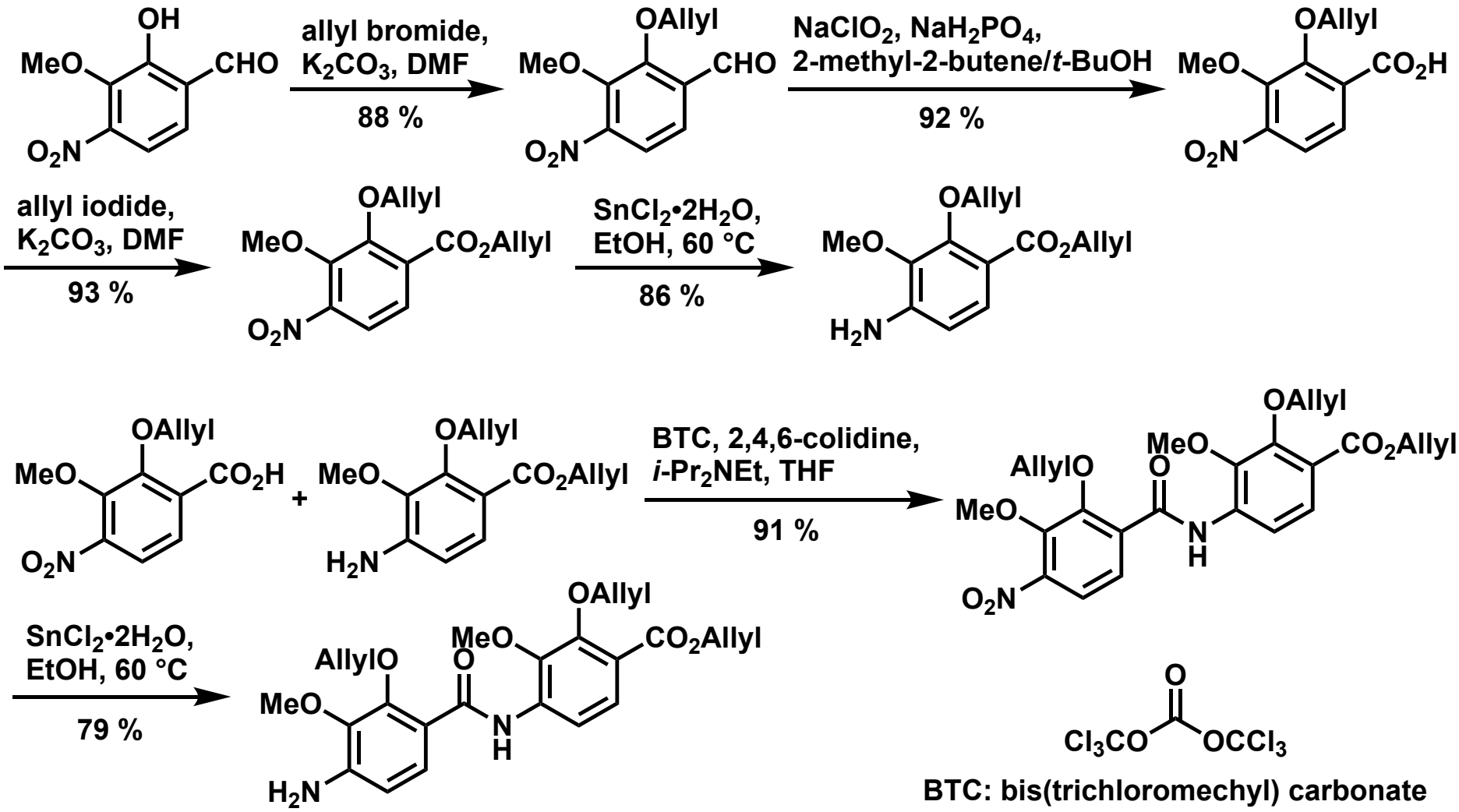


B-C-D unit



1) Krets, J.; Kerwat, D.; Schubert, V.; Grätz, S.; Pesic, A.; Semsary, S.; Cociancich, S.; Royer, M.; Süssmuth, R. *Angew. Chem. Int. Ed.* **2015**, *54*, 1969-1973.

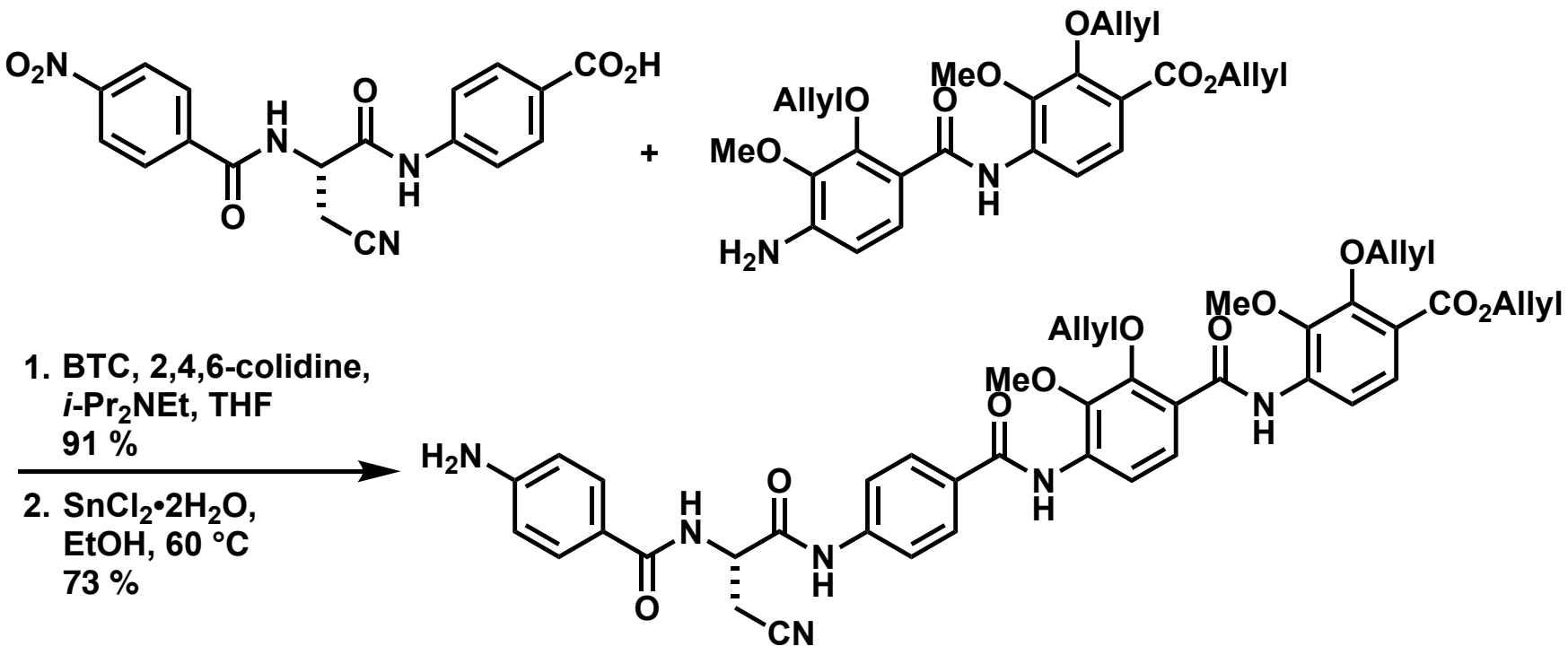
Construction of E-F Unit



BTC: bis(trichloromechyl) carbonate

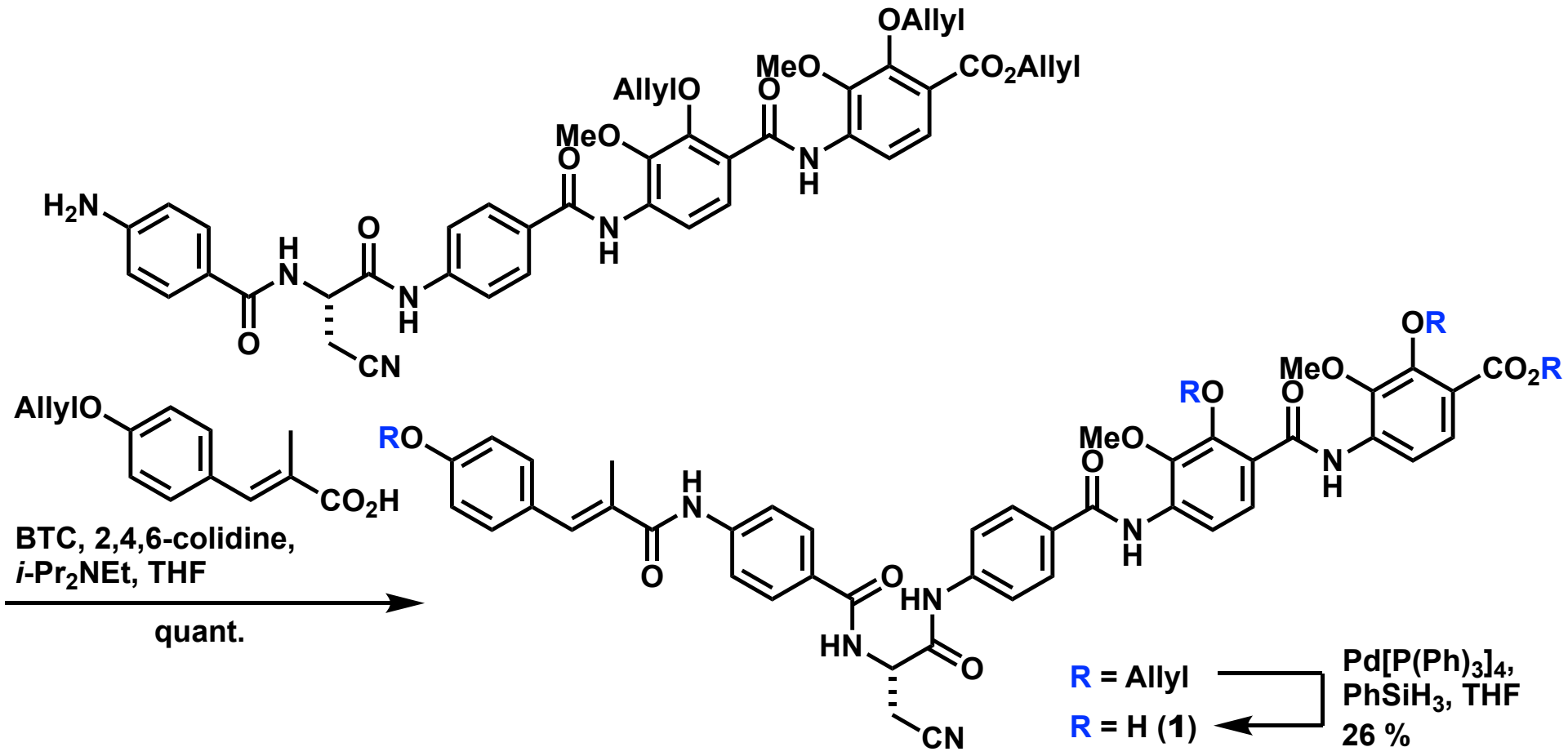
1) Krets, J.; Kerwat, D.; Schubert, V.; Grätz, S.; Pesic, A.; Semsary, S.; Cociancich, S.; Royer, M.; Süssmuth, R. *Angew. Chem. Int. Ed.* **2015**, *54*, 1969-1973.

Coupling of B-C-D Unit



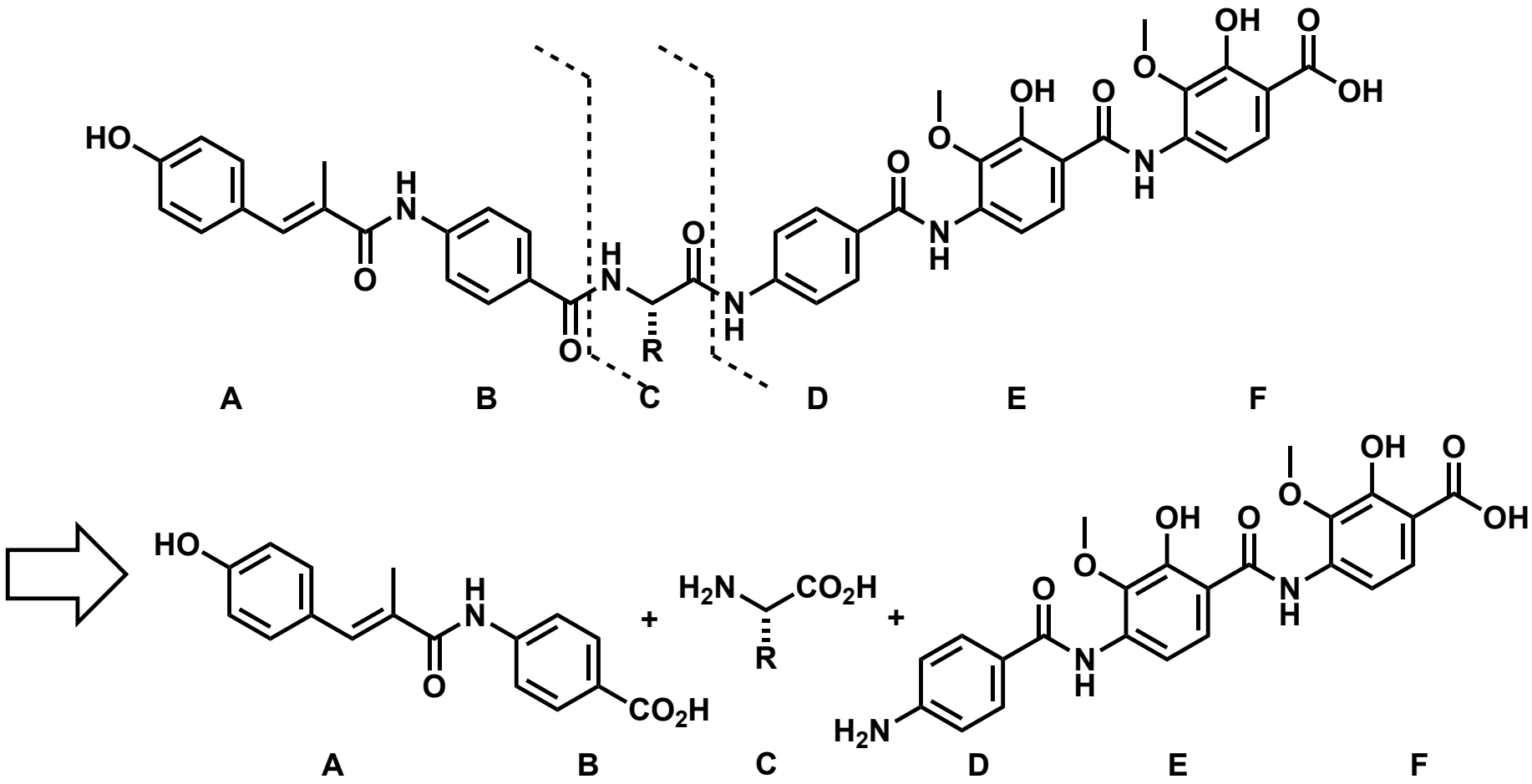
1) Krets, J.; Kerwat, D.; Schubert, V.; Grätz, S.; Pesic, A.; Semsary, S.; Cociancich, S.; Royer, M.; Süssmuth, R. *Angew. Chem. Int. Ed.* **2015**, *54*, 1969-1973.

Total Synthesis of 1



1) Krets, J.; Kerwat, D.; Schubert, V.; Grätz, S.; Pesic, A.; Semsary, S.; Cociancich, S.; Royer, M.; Süssmuth, R. *Angew. Chem. Int. Ed.* **2015**, *54*, 1969-1973.

Modified Synthetic Strategy for **1**



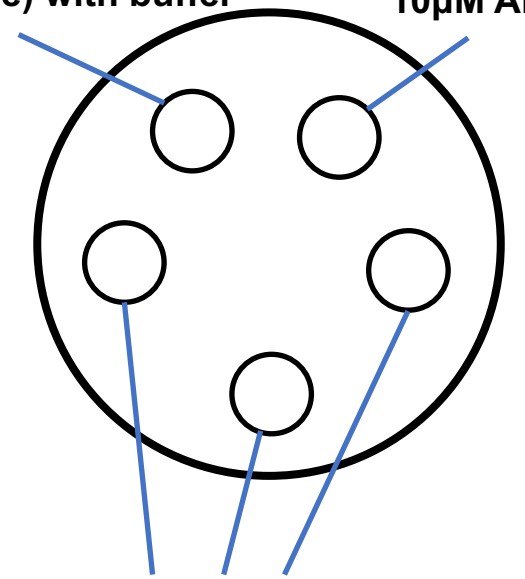
1) Krets, J.; Kerwat, D.; Schubert, V.; Grätz, S.; Pesic, A.; Semsary, S.; Cociancich, S.; Royer, M.; Süssmuth, R. *Angew. Chem. Int. Ed.* **2015**, *54*, 1969-1973.

AlbD Assay for Albicidin Analogues (2019)

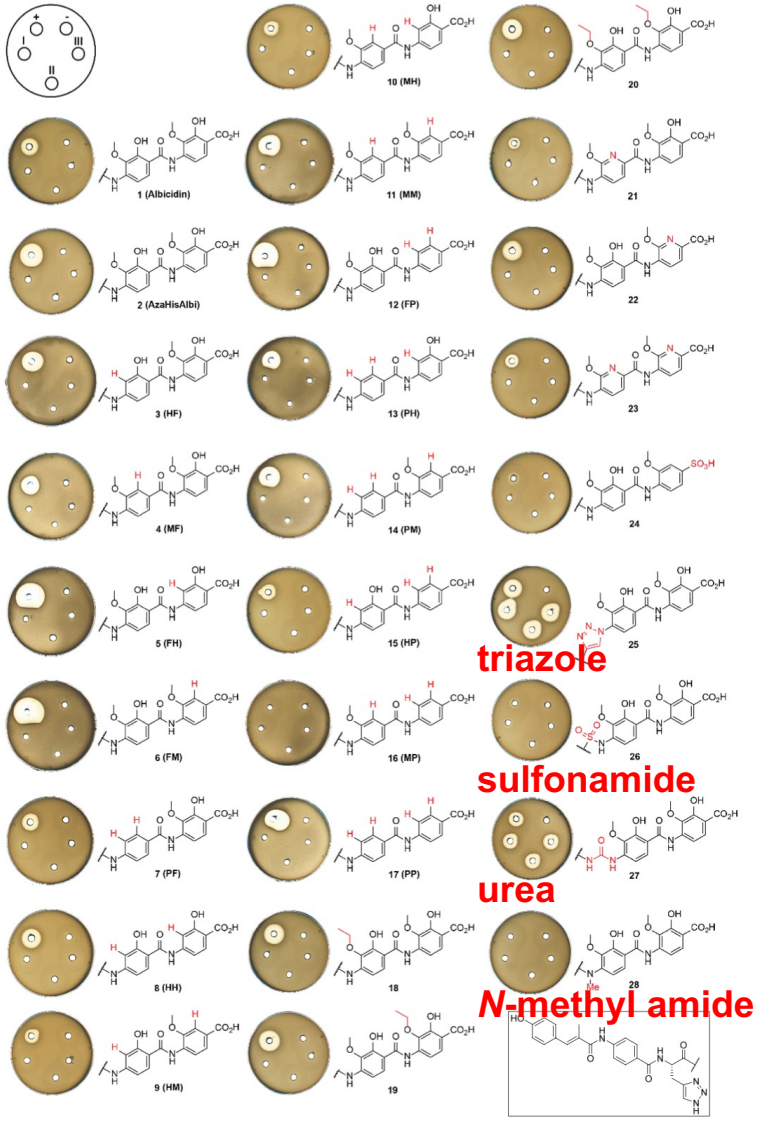
***E. coli* DSM1116 with LB agar**

Positive control:
100µM albicidin
(or analogue) with buffer

Negative control:
10µM AlbD with buffer



AlbD reaction mixture:
10µM AlbD and 100µM
albicidin (or analogue) with buffer



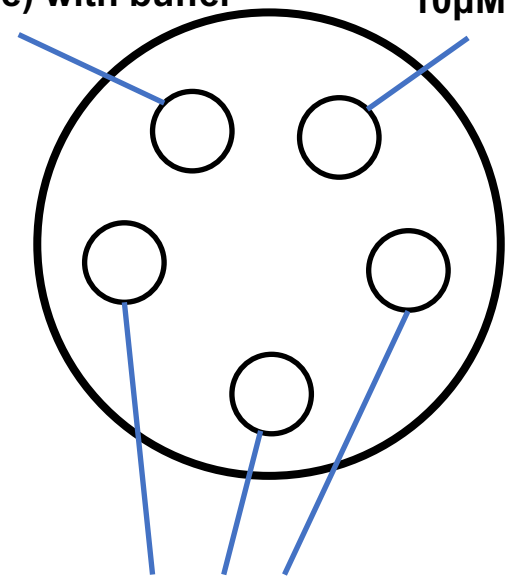
1) Behroz, I.; Durkin, P.; Grätz, S.; Seidel, M.; Rostock, L.; Spinczyk, M.; Weston, J.; Süssmuth, R. *Chem. Eur. J.* **2019**, *25*, 16538-16543.

AlbD assay for Albicidin Analogues (2021)

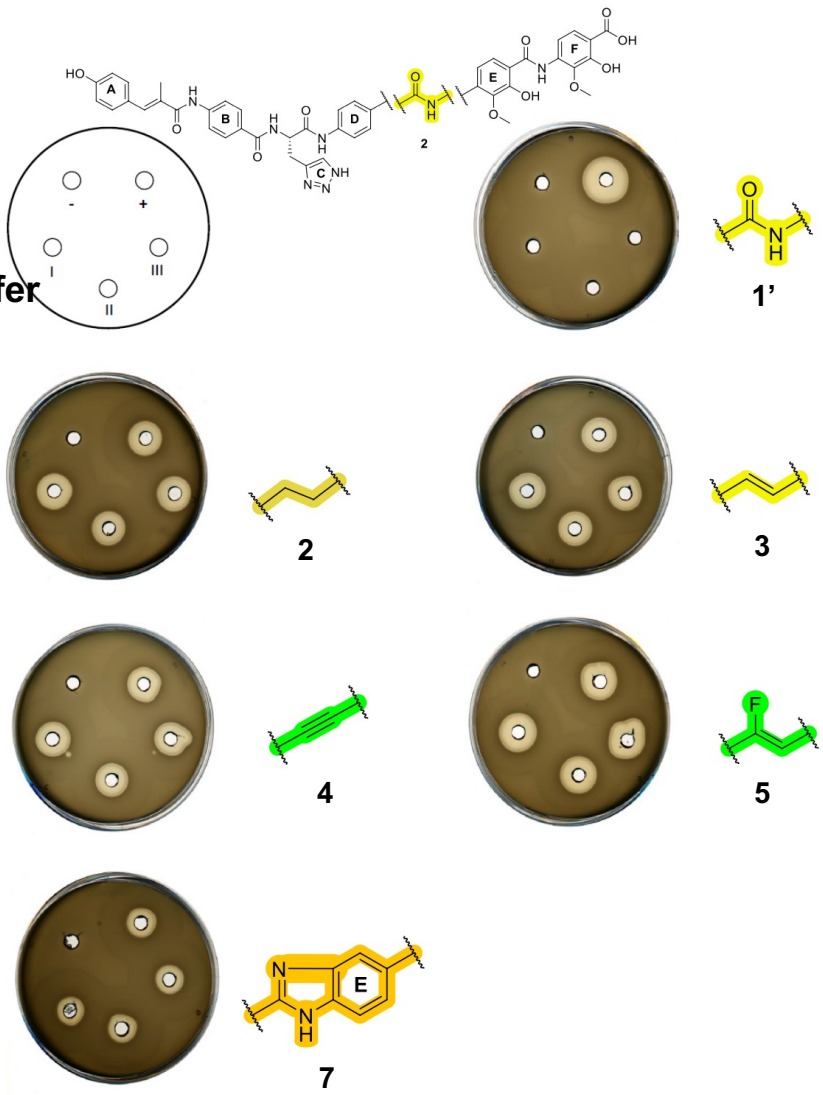
***E. coli* DSM1116 with LB agar**

Positive control:
100µM albicidin
(or analogue) with buffer

Negative control:
10µM AlbD with buffer



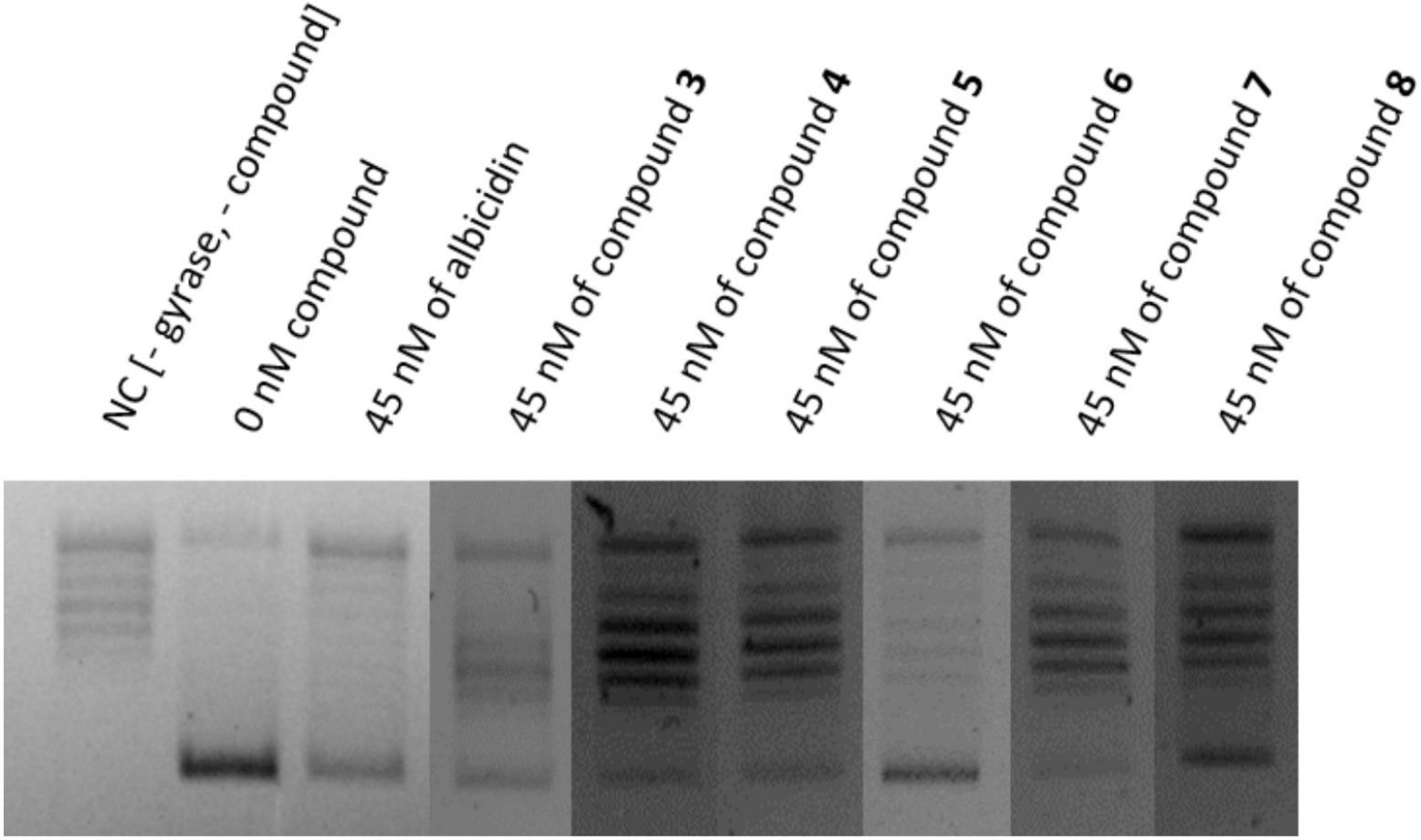
AlbD reaction mixture:
10µM AlbD and 100µM
albicidin (or analogue) with buffer



1) Kleebauer, L.; Zborovsky, L.; Hommernick, K.; Seidel, M.; Weston, J.; Süssmuth, R. *Org. Lett.* **2021**, *18*, 7023-7027.

E. coli DNA gyrase inhibition assay

relaxed pBR322 plasmid + *E. coli* DNA gyrase + albicidin or each analogue



1) Kleebauer, L.; Zborovsky, L.; Hommernick, K.; Seidel, M.; Weston, J.; Süssmuth, R. *Org. Lett.* **2021**, *18*, 7023-7027.